

nature*January 27, 1977*

Ford leaves a healthy legacy

GERALD FORD will no doubt be recorded in the history book as a President who, in his brief term of office, performed an extraordinary act of national healing, pouring a good deal of soothing balm on the political wounds inflicted by his predecessor. He did nothing too flamboyant, pursued few major new initiatives, and left the overall structure and operations of the federal government virtually intact. That pretty well sums up his record in science policy as well.

When he entered the White House two-and-a-half years ago, relations between the federal government and that slice of the population known as the scientific community were at an all-time low. When he handed over the reins to President Carter last week, the management and funding of federal science had been placed on a firmer footing, and the scientific community was, if not satisfied, at least mollified.

Mr Ford's most conspicuous act of healing in science affairs was the reinstatement of a science policy office in the White House, a move which has won much praise even though the new office came into being a scant five months before Mr Ford left Washington. Few of Mr Nixon's acts caused as much bitterness among scientists as his decision in 1973 to abolish the Office of Science and Technology and the President's Science Advisory Committee, bodies which had been providing scientific advice to the White House for more than a decade. Mr Ford's decision to reinstate a science advisory apparatus in the White House, though it took most of his Presidency to accomplish, has laid the basis for more rational scientific planning.

As far as budgets for research and development are concerned, Mr Ford has again placed matters on a relatively sound basis. When he came to power, he inherited a dismal state of affairs. During the previous few years, total federal support for research and development had declined as inflation more than gobbled up the small dollar increases proposed by Mr Nixon. Basic research was especially hard hit, with federal support declining in real terms by more than 20% between 1969 and 1973. And superimposed on the slow downward drift was a debilitating series of abrupt oscillations in funds for some programmes as Mr Nixon attempted to put a hold on public expenditure and to shift funds into priority areas at the expense of less politically favoured programmes. During Mr Ford's brief Presidency, there has been some real growth in funds, and basic research has been given a welcome boost.

The growth was particularly surprising because the three budgets which Mr Ford submitted to Congress all reflected his overriding concern to curb the overall rate of growth in the federal budget; science was given a

financial boost while other programmes, particularly those in health and welfare, were held back. And Mr Ford's last budget, delivered to Congress just three days before his departure, continued the trend and even added some long overdue support for new research programmes in agriculture and the earth sciences.

But that does not mean Mr Ford has solved all the problems in federal support of research and development, and that he is handing President Carter a trouble-free scientific enterprise. Far from it.

Energy policy is in a mess, and oil imports are actually higher now than they were before the 1973 embargo. The overall strategy which has marked both the Nixon and Ford energy policies was to throw massive amounts of money into energy research and development, particularly nuclear technologies, in the hope of increasing energy supplies. Little attention has been paid to curbing demand, either through regulation or research on energy-saving technologies, and President Carter has rightly criticised the outgoing Administration for its failure to establish a coherent energy policy. A particular failure has been the Ford Administration's attempts to curb nuclear proliferation, where policies have been thrown together in response to crises rather than developed by careful planning.

In the area of biomedical research, Mr Ford inherited a particularly dismal situation, and though he has made some progress, he is leaving his successor a number of problems. Two chief factors have led to disarray in biomedical research planning. The first was the passage of the National Cancer Act, which has caused funding for cancer to balloon while support for many other areas of research has languished. Although Mr Ford has tried to redress the balance in his budgets, he did not acknowledge, let alone implement, some modest recommendations for reform published last year by a Presidential commission.

The second principal area of irritation has been a continuing struggle between Republican Administrations and the Democratic Congress over the size of the budget for the Department of Health, Education and Welfare. Presidential vetoes and refusals to compromise on both sides have greatly delayed the passage of appropriations bills and caused federal funds to flow into the National Institutes of Health in a series of surges and trickles. Mr Ford's budgets, including his final proposals, have perpetuated the trend.

Nevertheless, on balance, Mr Ford is leaving his successor a surprisingly healthy scientific legacy, and few major changes in the federal science policy should be anticipated from the new Administration, at least in the next year or so. □



Science as an education

In this shortened version of a talk he gave recently on BBC Radio 3, Professor Sir Hermann Bondi, Chief Scientific Adviser to the Ministry of Defence, argues for a view of science as an education of the mind, rather than as professional training

My thesis is that science should be taught like the classics but has increasingly been taught like medicine. A medical education is a professional training with a practising doctor as end product. A classical education (and I would include more than just the classics here) has no such clearly defined end product, but is widely accepted as a good preparation for demanding tasks in administration and management. The reason is that students are taken through the thinking of some of the finest minds humanity has ever known, discuss and debate the difficult, often undecidable questions that these people discussed, and are trained to express themselves by word of mouth as well as by writing and to give a structure to their ideas, however complex or sophisticated the subject.

This is precisely the ability helpful in any senior task; in such a task one always deals with people and it is through argument and discussion, papers and analysis that one arrives at the kind of conclusion, at the kind of leadership, at the kind of consensus this necessary for achieving anything in administration and management. The only criticism must be that classics courses are very short indeed on numeracy, a skill which is of particular importance to administrators and managers who need to analyse statistics critically.

We have had a lot of trouble in recent years with young people not coming forward in the numbers expected to do science. This limited success of science in the all-important field of marketing an academic education is often ascribed, rightly, to the liking of young people to work with people rather than to deal in facts. This attitude reveals a misconception of what science is like, that to me is simply staggering. Science doesn't deal with facts; indeed, fact is an emotion-loaded word for which there is little place in scientific debate. Science is above all a cooperative enterprise. It is a social activity which is perhaps more successful than any other in enabling people of different religion, politics, race, culture and ideology to work together, and it is thus a particularly human subject.

Science reveals its humanity most in

those research issues that are the subject of current debate, discussion and seminar. In these fields there is no certainty, only arguments. These arguments can indeed be supported in an exceptionally important way by experiment, but can also be disproved by a sufficiently careful and ingenious experiment or observation. Science is thus a field in which imagination is of the utmost importance; not, it must be emphasised, untrammelled imagination, but imagination carefully controlled by what can and cannot be tested experimentally.

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When we look at science, not so much as a profession but as an intellectual adventure, it is clear that it has had some of the finest minds that mankind ever produced, grappling with problems a little harder to define than some of the philosophical problems of the ancients, but also problems where, through the test of experiment and observation, human imagination has been brought to its highest peak. This aspect of science does not come across anything like well enough to attract young students.

If it is educational for performers of senior and responsible tasks to be taken through the thoughts of a Plato, is it not just as good, perhaps even better, for them to be taken through the thoughts of a Newton or an Einstein? I say better because of the additional demands of numeracy. I claim that if we conceive of science as a group of subjects offering an education in thinking, communication and particularly the evolution of human ideas generated by an unequalled creativity, originality and imagination, then the education that can be provided

is unsurpassed.

Hitherto the voracious appetite of government and industry for scientists during the 1950s and much of the 1960s propelled the universities into producing the maximum number of professional 'bench' scientists they could, which was thought, to some extent at least, to involve stuffing as much scientific knowledge into them as was possible. I would not wish it to be thought that I view those efforts—in some of which I partook—as bad or disadvantageous; only that the emphasis was confined, incorrectly, to the aim of producing professional scientists.

If one had viewed science as an education of the mind rather than as professional training, naturally students emerging with a first degree would have known less science. But they would have known more about the kingdom of scientific ideas, about how to argue, about the evolution of human thinking and about how scientific debate and the interaction of experiment and theory stimulate creativity.

Further, such an orientation of science courses might also produce better scientists, first because such teaching would attract many of the ablest young people now repelled by the supposedly inhuman face of science, and second because in science itself an ability to argue, debate, and understand the other person's ideas, however imperfectly expressed, is at least as important as sheer scientific knowledge.

It ill-becomes scientists to deplore the fact that so few of them are in parliament, the upper reaches of the Civil Service, the banks, the highest positions in industry. For have science courses been aimed to produce such people? There have been a number of efforts in the past ten or fifteen years to improve the balance. They have had most creditable success in establishing novel courses, but these only affect a very small proportion of the science students and therefore do little to alter the prevailing misconception of what science is.

I do not want it to be thought that science as an education is intellectually less demanding than an education in science. Quite the contrary. And I certainly would not wish to propagate a course that was not really demanding, because that would fail in its purpose: attracting the ablest young people to what is the most exciting area of human enterprise. □

Asia's marginal lands

Robert Orr Whyte examines the prospects for reclamation of an important natural resource

There are no unproductive regions. There are only poor methods for cultivating land; there is no such thing as poor land. Provided only that people manifest in full measure their subjective capacities for action, it is possible to modify natural conditions.

Mao Tse-tung

IF the countries of monsoonal and equatorial Asia are to act upon the injunctions of United Nations and bilateral agencies and learn to feed themselves, they must utilise all their land and sea resources to the full. At present, there is insufficient knowledge of the real nature and magnitude of the land resources to show whether and to what extent this enormous target can be met. An assessment of land resources on modern lines is essential to confirm or adapt the existing land classes, and to define their relative areas and potential. The classes usually recognised are forests (protective or productive), grazing lands, uncultivable land other than forests, and cultivated land, whether used under systems of shifting cultivation, for plantation agriculture, or for dryland or irrigated crop production. However, one major land class which does not regularly appear in the statistics is marginal land.

Definition of the term depends upon the discipline of the speaker. Most of the uncultivated land, and much of the cultivated land that one sees in flying over the South Asian subcontinent or over the People's Republic of China, is marginal in nature. The agriculturist regards land as marginal when, because of terrain, soil or climatic conditions, the cultivation of arable crops becomes a risky enterprise, involving complete or partial crop failure in two or more years out of ten. The forester regards land as marginal when he cannot apply the appropriate silvicultural techniques to stands of natural vegetation, or when he cannot plant, maintain and harvest the produce from reforested blocks. Grazing land, particularly in arid and semi-arid regions, is marginal when it cannot supply feed of adequate quantity and quality to nomadic and migratory flocks and herds within a radius of daily walking distance, or when resources for stock watering are inadequate.

Land may be marginal in its natural state, or may have been rendered marginal due to the activities of man. The

total area of marginal land of all types is not known. It is probably at least as large as the area under non-marginal cultivation at present. Only part of the marginal land of the region comes within the scope of the UN Environment Programme's Conference on Desertification, to be held in 1977.

It is debatable whether the greater potential for reclamation and increased production does not lie in the more densely populated countries of humid tropical Asia. The final criterion must be economic—whether the expense involved in bringing land back into economic use or in raising it from one plateau of production to the next above for the benefit of the local population can be justified under present conditions.

Attention to the problem of marginal lands must also be seen in correct perspective. It takes second place to the increase of productivity on existing cultivated land, which remains the great potential. The world average production of cereal grain per hectare is 1,898 kg. If the standards of Japan and other developed countries were to be achieved, the world output of cereals from existing cultivated land would be 3,286 million metric tons, or 859 kg per capita, or 95% of American consumption (see Lincoln Gordon and C. A. Pryor, *Science*, September 19, 1976). Yet we are still losing ground in this respect. In India, net availability per capita per day of cereals and pulses from domestic production was: 1956, 15.2 oz; 1974, 15.97 oz, and 1975, 14.65 oz.

Area increasing

The shifting cultivator has made the largest contribution to the man-made area of marginal land, and the area is still increasing rapidly year by year. Increased pressure of population on existing land resources and the progressive loss of traditionally exploited forest land to settled cultivators and timber extractors cause progressive reduction in the period of bush fallow. Regeneration of vegetation does not occur between successive periods of cultivation, and soil fertility deteriorates. What remains in humid tropical South-East Asia are pure stands of *Imperata cylindrica*, the 'green cogon deserts', much of which may still be reclaimed and can be regarded as marginal.

An estimated 2.5 million hectares of potentially productive land are lying barren, because of high sodicity (alkali

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Turning poor soil to farmland

or sodic soils), in the west of the region and in the Indian States of Punjab, Haryana and Uttar Pradesh in particular. Inadequate surface drainage and consequent stagnation of water for prolonged periods, and a rise in the ground water table and accompanying physicochemical changes, appear to have resulted in the formation of these soils. These areas are currently grazing grounds for domestic livestock of low productivity or for abandoned bovines which have reverted to a semi-wild condition.

Probably at least comparable in area to the marginal lands created by shifting cultivation are the areas abandoned after deforestation. This happened in historical times in the Himalaya and their associated ranges and in China, and in recent and modern times in the Philippines, Indonesia and East and West Malaysia. Only limited areas have been reforested or transferred to plantation and arable agriculture in new economic ecosystems. These, however, rarely have the same conservation value as the biological ecosystems they have replaced.

The slaughter of the tropical rain forest in South-East Asia is eliminating the last remnants of a unique biological ecosystem, with its relatively unexplored genetic resources of plants and animals. And the areas from which marketable timber have been removed are left in such a condition after the operations of logging and extraction that further deterioration is inevitable, in terms of plant cover, soil erosion, desiccation of the environment and increased carbon dioxide content of the atmosphere—all factors not so far assessed accurately.

South Asia's marginality

The establishment of the drought-resistant plant and animal communities in the South Asian subcontinent, formerly tropical rain forest, followed the

creation of the monsoonal climate. This may in terms of land productivity be regarded as a marginal ecoclimate. The already marginal potential of most of the subcontinent became affected by the arrival of the Aryans with their browsing and grazing animals, their use of fire, and later of iron tools. The ecosystems in South Asia, with which the modern planners of use of arid and semi-arid lands have to cope, have been further reduced in potential in recent and modern times by the great increase in human and livestock populations, the latter due largely to politico-religious factors beyond the control of the land scientist.

In a region where the amount of arable land per person is estimated to have been 0.36 ha in 1950, 0.30 ha in 1965, 0.24 ha in 1975, and to be 0.19 ha in 1985, it is realistic to propose that any major developments in animal husbandry will be limited to marginal land which cannot produce cereal and legume grains for direct human consumption. In terms of the grazing and browsing resources of South Asia alone, the botanical composition of plant covers of the arid and semi-arid lands has deteriorated from a condition in which the superior perennial grasses provided reasonably good feed to livestock in the monsoon and subsequent months to less valuable grass covers of inferior perennials and annuals.

In the East, especially in Japan, Taiwan and China, there are large areas of sloping land, originally under a forest type in conditions between lowland monsoonal and upland temperate, which have been rendered marginal in terms of forest production and agriculture.

Matter of concern

To the conservationist, the ever-increasing area of land being rendered marginal is a matter of profound concern. Idealistic proposals for the total conservation of the environment are difficult to maintain with the population/land position in large parts of Asia. Marginal land *per se* generally represents a continuing and frequently increasing danger in terms of its own conservation, and of the conservation of soil and water on lands lower in the same catchment not yet rendered marginal. A realistic plan for conservation would embrace the preservation of natural biological ecosystems wherever this can be socially and economically justified. It would cover the reclamation of marginal lands either to their original condition or to a new balanced economic ecosystem in which sustained soil fertility and productivity can be maintained by the application of appropriate measures for conservation.

either direction has so far been a matter of economics. The rural people responsible for making land marginal have had neither the knowledge nor the financial resources necessary for its correct treatment. Governments of Asian countries in which the return of marginal land from an unproductive to a productive state would be of great economic importance have not approached the problem with the energy it demands.

Again, this is largely because they apply the principles of conservation economics, of return upon capital investment, cost/benefit, input/output ratios and so on. But if most of the countries of Asia are at the age where it is the economics of survival which apply, a different set of criteria will be necessary if measures are to be applied on a sufficient scale.

The administrative structure of most Asian countries does not permit them to emulate the People's Republic of China, where in the winter of 1975–76 130 million rural workers were mobilised for what BBC monitors translated as farmland capital construction projects. These ranged from the reclamation of desert grasslands of Inner Mongolia and Sinkiang and the reforestation and levelling of land for irrigated cultivation in the eastern provinces, to the terracing of mountain slopes in the humid tropical island of Hainan.

Elsewhere some research is in progress. Increased productivity of marginal drylands is an important objective of the Indian government in association with the Canadian government, and at the International Centre for Research in the Semi-arid Tropics (ICRISAT), in Hyderabad. During the past six years the Central Soil Salinity Research Institute at Karnal, Haryana, has evolved economic and speedy methods for the reclamation of alkali soils of the Indo-Gangetic plains.

The essential components are land

levelling, the application of gypsum or other additive, the application of fertilisers including micronutrients, the selection of suitable crops and crop varieties, and the adoption of suitable management practices. Semi-dwarf, high-yielding varieties of rice are ideally suited in the *kharif* (rainy) season, to be followed by wheat in the *rabi* (winter) season. Large-scale reclamation programmes based on this research are now being launched to reclaim lands which have been lying uncultivated.

Delaying action

Foresters in the region are conducting a delaying action against rival claims on land for agriculture or for the grazing of livestock. The tropical rain forests present a major problem. Foresters still talk of the promotion after logging of natural regeneration on 100-year cycles, but this is rarely followed in practice. In Java, the relict vegetation on denuded land which might theoretically be reclaimed is used for fuel collection by thousands of people who have few alternative sources of income. Claims are made for the control of *Imperata cylindrica* by deep ploughing (Sumatra), replacement by sown fertilised pastures correctly managed to prevent regeneration (Sabah and the Philippines), or the use of systemic selective weed killers (Malaysia).

The marginal nature of the grazing and browsing resources of arid and semi-arid India are the concern of three research institutes, the Central Arid Zone Research Institute, the Indian Grassland and Fodder Research Institute and the Central Sheep and Wool Research Institute. These compare the relative merits of slow but cheap ecological progression and of reseeding with species of the local climax covers. A substation of the Japanese National Grassland Research Institute at Nishinasuno is developing methods for hill slope utilisation by the cheap method of hoof cultivation for the rearing of Holstein heifers for the dairy industry—a technique that may well be applicable in mainland China, Korea and Taiwan.

The reclamation and planning of future use of marginal land fall between a number of departments and institutes of central or state administrations. If these were to be regarded as a discrete aspect of land use the economic benefit would be great, particularly for the livestock and forest industries. That some type of joint action on a regional basis would be profitable, through ASEAN, the Asian Development Bank or the UN International Consultative Group for Agricultural Research, seems almost indisputable. □

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Terraced fields in China

CANADA

Reorganisation in Ottawa

A long-awaited bill on governmental organisation of scientific activities was finally presented to the Canadian Parliament shortly before Christmas. David Spurgeon reports from Ottawa.

THE bill proposes the reorganisation of the federal granting councils which provide funds for university research, and makes other changes concerning the Science Council of Canada and the Defense Research Board. At the same time Hugh Faulkner, Minister of State for Science and Technology, responded to concerns of the community about falling financial support, and said consideration is being given by the government to providing increases in 1977-78 budgets to cover inflationary increases in the cost of doing research.

The granting council changes were recommended several years ago by the Senate's special committee on science policy (the Lamontagne committee) and changes were promised by the government in February, 1974. According to Mr Faulkner, the bill is "the culmination of a lengthy series of studies, reports, meetings, and debates on the subject of science policy . . .".

The bill creates a Social Sciences and Humanities Research Council, leaving the Canada Council, which has supported both the arts and research in the social sciences and humanities, to deal entirely with the arts. Mr Faulkner said the reorganisation "reflects the growth in size and quality of the social sciences and humanities (in Canada) and the new perception of their importance to the attack on socio-economic problems, to national sovereignty, and to our cultural development". The bill also creates a Natural Sciences and Engineering Research Council, which will remove from the National Research Council of Canada the function of granting funds for university research in these areas. The NRC will henceforth deal with its own laboratories, industrial R&D support programmes, and a national programme of scientific and technical information. Mr Faulkner said the new council "recognises the need for a separate agency to support university research in these disciplines (natural sciences and engineering) and to foster improved dialogue between natural scientists and research engineers."

The Medical Research Council Act is amended under the new legislation to remove the restriction preventing its support of research in public health,

but otherwise the council remains unchanged.

In order to advise and co-ordinate the work of all the granting bodies, Mr Faulkner will establish an Inter-council Co-ordinating Committee, chaired by the Secretary of the Ministry of State for Science and Technology. The committee will include the presidents of the granting councils and will report to Mr Faulkner.

Although the criterion of excellence will continue to be the basis of university research support, additional funds will be provided to the councils for regional balance of scientific capability, national problems and support of inter-disciplinary research.

The Science Council's mandate is also restated in the legislation, to provide it with a greater national and public role. It has actually played such a role for years, but, according to the Minister, "the new mandate . . . will state clearly that the Council is to act as a source of public information and to provide advice on the profound effects developments in science and technology will continue to have on our way of life."

Amendments are also proposed to the NRC Act to give it flexibility to respond to national problems more readily when science and technology have a part to play. Provision has also been made to transfer the responsibility for Canadian Patents and Development Limited from the NRC to the Ministry of Industry, Trade and Commerce. This is supposed to make easier exploit-

ation of inventions from government and university laboratories by industry. Thus the role of NRC is to change somewhat, with "less responsibility for the general development of science in Canada and more for using the demonstrated capability of its laboratories in tackling national problems."

● Towards the end of last year, the Lamontagne committee chairman, Senator Maurice Lamontagne, reported on progress to date in the committee's resurrected hearings, and the group resolved to go on and finish them in 1977. Senator Lamontagne said the current sessions have heard from 31 organisations and have accumulated about 1,300 pages of evidence. Six or seven more meetings are needed to conclude the hearings, which are concentrating on determining how many of the recommendations the committee made in 1972-73 have been followed by the government, and in what way. Mr Lamontagne noted that the current hearings have shown that many recommendations have been carried out, but that failure to follow some "is now producing what has been described to us as an acute crisis, endangering Canada's future capability in the whole field of scientific research, technological development and economic and social innovation." The two recommendations he noted had not been followed concerned the need to transfer older researchers to other jobs in the private and public sectors and the need for greater national expenditure on R&D. If the present situation continues, he warned, "Canada may lose a whole new generation of researchers". □

REACTION to the bill was not uncritical. Bill Kempling, Progressive Conservative MP for Halton-Wentworth, asked whether it was the first of several steps to eliminate the science ministry. He noted that MOSST can be cancelled at any time, without reference to Parliament, by an order in council. And he suggested that what the bill really does is increase the number of accounting, auditing and administrative personnel at a time when the government is talking about restraint in spending.

Mr Kempling noted that the inter-council co-ordinating committee has no budgetary control and called it "another layer of bureaucracy." It would have been better to have set up an overall science foundation reporting to a full minister, with a number of divisions for different disciplines.

Cyril Symes, New Democratic MP from Sault Ste Marie, said it appears

to him that whenever the Liberal government gets into trouble and has no real policy answers, "it carries out the old double shuffle: it reorganises parts of its bureaucracy with great fanfare to make it appear that it is doing something, whereas in actuality it is just shuffling the cards . . .". Mr Symes suspected that the bill was a sop to the lobbying of the science community, which had shown concern about funding cutbacks.

Paul Yewchuck, Progressive Conservative MP from Athabasca, was concerned about the chairmanship of the inter-council committee: "I can think of no good reason for the minister wanting one of his bureaucrats chairing the . . . committee, unless he thinks he can control the actions of that committee better if one of his underlings is in charge of it . . . I would rather see an active scientist taking this position."

IN BRIEF

Scientific freedom

A group of Swedish scientists has recently formed a committee for scientific freedom which is to agitate on behalf of persecuted or harassed scientists in the same way that Amnesty International works for political prisoners. About 100 scientists have so far shown interest in the committee, which is chaired by Professor Ragnar Romanus of Gothenburg. The idea is that physicists will work on behalf of physicists, biologists for biologists, and so on.

The scientists say that their work will not in principle be directed at any particular countries. Several of the committee's members have previously urged the Soviet authorities to grant exit visas to Jewish scientists wanting to emigrate to Israel. They stress how-

ever that they do not want to condemn any political system as such, but rather the treatment that various systems mete out to their scientists. At the moment, Argentina is an obvious target for their work.

Hot summer, good news

Infant mortality figures for England and Wales in 1976 show a marked improvement on figures for earlier years, and some of this improvement can probably be attributed to the long hot summer. Improvements in obstetric practice and care of newborn infants must also have had an impact, however, in the overall drop in mortality reported by the Office of Population Censuses and Surveys.

Post early neonatal mortality (one week to one year in age) regularly

shows a seasonal term, but in 1976 this was exceptionally pronounced, summer deaths being 4.1 per thousand (annual rate) compared with 7.3 for the winter quarter. This helped in large part to cause a drop in the whole year's figures by an amount almost equal to the drop for all the previous five years.

Perinatal mortality (28 weeks after conception to one week after birth) shows a much smaller seasonal term, but there too substantial gains have been recorded. The figure for 1970 was 23.5 per thousand; by 1975 it was down to 19.3 but in 1976 the figure dropped to 17.5.

Infant mortality is now about half the level it was in the early 1950s, but until this past year many people have expressed concern that Britain's performance in reducing the rate did not match that in other developed countries.

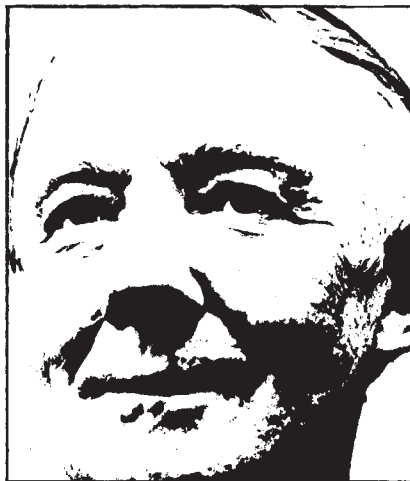
A FEW years ago politicians were saying that British scientists were about to launch the "white hot technological revolution" which, fanned into flames by the efforts of the civil servants in a newly conceived Ministry of Technology, would revitalise British industry. The revolution, unfortunately, was stifled at birth, and the new Ministry soon disappeared.

Today Britain's scientists are blamed because the growth of industrial productivity is slow or non-existent. They are castigated because they appear to be unwilling to follow careers in industry, or to direct their research into fields which might ensure economic progress. University courses for undergraduates and graduates are criticised for not imparting the proper skills, and even more for not giving the necessary "motivation". The country's most prestigious committees debate these subjects interminably, but have so far made no suggestions for improvements in education, training or organisation which engender any confidence in academic or commercial circles.

Yet thirty years ago British scientists enjoyed, and may even have deserved, greater prestige. It was generally agreed that they had made many remarkable contributions of a very practical nature to both military and other problems (medicine, public health, transport for instance) during the Second World War. They showed themselves able to tackle and solve difficult and important problems often far removed from those they had previously studied. This they did for the most part with the minimum of expensive and complicated equipment,

and often at very little cost to the community. This gave rise to the belief that if Britain increased her scientific establishment many fold, and if its financial support was allowed to grow exponentially (conditions ful-

Effective science



KENNETH MELLANBY

filled during more than twenty post-War years), she would solve many of her social and economic problems.

Unfortunately the results have been disappointing. Today all is not well with both science and industry in Britain and in many other parts of the world, and most attempts to diagnose and cure this malaise have been unsuccessful or have made things worse. Surely the obvious course would be to examine previous successes, and see what can be learned from them. For instance were there any common

factors in their education, training and experience which made war-time scientists so adaptable and successful at solving so many of the immediately important problems which faced them and the country?

It is dangerous to generalise, but it is clear that many of those who were most successful in war-time science had previously worked only in university departments or for research councils. Their 'training' had been entirely academic, and, up till then, they had mostly worked in their own way on problems of their own choice. As far as I know, none had ever attended a course in 'management'. In many cases it was the scientists themselves who first became aware of the existence of the problems which they eventually tackled. They often had to convince the authorities, including the expert committee (which existed even then, but fortunately in much smaller numbers than today), that the work was worth doing. And it was often the academic scientists themselves who took the successful initiative in ensuring that their results were put into practice.

In many ways the problems facing the world today are as serious and urgent as those which faced Britain during the war. Can anyone explain why scientists no longer operate as effectively as they did at that time? There are far more trained research workers, and, notwithstanding recent cuts, far more money is spent on science. Surely this is what our committees should be investigating, even if they discover that it is often their own existence which is the barrier to scientific and economic progress?

news and views

Sea surface temperature and climate

from P. B. Wright

SEA surface temperature (SST) is an important factor in climate. The physical reasons are readily apparent; if relatively cold and unsaturated air blows over the sea, heat and moisture are transferred from the sea into the air, and the rates of transfer depend on the temperature difference. Thus subsequent behaviour of the airstream depends on the temperature of the ocean surface over which it has passed; for example, the latent heat from the additional moisture evaporated into the air may be released into the upper troposphere at some distance downstream, modifying the circulation there. This may in turn influence the circulation even farther afield.

Moreover, SST has high thermal inertia. The sea surface takes a long time to cool down (or warm up) because any change of temperature has to be distributed through the top 100-m layer which is well stirred. Some of the effects of these properties are familiar—maritime climates are more equable and more humid than continental ones, and their seasons are delayed a month or two. It is not surprising therefore to find that SST variations play an important part in causing the climate to differ from year to year. A number of correlations have been discovered over the past few years—between SST near Newfoundland and atmospheric circulation over Europe, SST over the North Pacific and weather over both North America and Europe, and between SST in the Equatorial Pacific and the 'Southern Oscillation', which involves rainfall in Australia and many other regions.

Markham and McLain (see this issue of *Nature*, page 320) have now found a new example, a close relationship between SST in the equatorial Atlantic and rainfall in north-east Brazil. Correlation coefficients reach the

remarkably high value of 0.8 in four separate months. It is particularly noteworthy that correlations of 0.7 occur in a predictive formula—between December SST and the following January-to-March rainfall. Moreover they are highly significant, indicating a real physical relationship. The details of the chain of interactions are still far from clear. Warm SST probably favours a modified trade wind pattern, which affects the rainfall. But the physical basis is sufficiently firm, and the relationship sufficiently close (even after allowing for sampling variations), for useful forecasting of the rainfall in this drought-prone region of the globe to be possible. When the physical details become better understood, and the most relevant sea area is pinpointed accurately, improved forecasts should be possible.

The forecasting potential depends essentially on the fact that SSTs in the equatorial Atlantic not only influence the rainfall, but also persist for several months. This is similar to the behaviour of equatorial Pacific SSTs. This raises the following questions. Are there other influences of the Atlantic SST similar to those of the Pacific? Are the two regions in any way coupled? Why do anomalies persist so long, and why do they eventually change? It will be some time before these questions are answered. But another question comes to mind. Are there any other regions where SST anomalies tend to persist for several months or longer? If so, then whatever their cause, we ought to know of their existence, because they would imply a forecasting potential for regions whose climates they influence. This should be simpler to answer; it merely requires a routine statistical analysis of SST data series.

However there are serious problems with data, some of which are mentioned in the paper. It is a tedious and in-

tricate job to transform millions of observations from ships' logs into series of monthly means, checked for errors, for 5° squares over the globe. But the need for reliable homogeneous series cannot be overemphasised. Work is now in progress in the Meteorological Office at Bracknell to devise an objective procedure for identifying errors, which will be a valuable contribution to the task. Nevertheless, as this paper shows, even the data available at present "form a picture that is coherent and of predictive value".

The paper also looks at correlations between equatorial and higher-latitude climates, and suggests a plausible mechanism by which warm South Atlantic sea temperatures could favour a deeper upper-air trough near Newfoundland. This sequence of interactions is the reverse of one put forward by Namias some years ago. The problem of cause and effect is a thorny one. It will not be answered from simple correlation studies like that of Markham and McLain, but these are valuable in providing pointers. Numerical models will have a crucial role; one could impose a persistent SST anomaly and look at the effects in different latitudes. Some such experiments have been made on both the Pacific and the Atlantic, which clearly indicate that equatorial SSTs can greatly influence higher-latitude circulation patterns.

So there is a great deal of further work needed along several parallel lines. The SST data base needs to be improved and made available. The homogeneous series so obtained should be analysed to discover areas where persistence is strongest. These areas should be used in correlation analyses to discover regions for which forecasts are possible, and also in numerical experiments to investigate the physical relationships which underlie the correlations. □

A marine biologist at Pompeii AD 79

from T. E. Thompson



Pompeii Exhibition

THE Pompeii AD 79 exhibition at the Royal Academy contains, in a mural on loan from the Naples Museum (number 120177), a mosaic illustration about 90 cm square which shows fish and other marine animals of culinary importance from the Bay of Naples. These organisms are portrayed with such vigour and scientific accuracy that marine biologists may well find this mosaic the most interesting and challenging of the exhibits. The artist was

a scientific illustrator of rare talent making this composition, originally executed as a domestic decoration, well worth closer inspection.

All the animals shown in the mosaic were primarily edible, with the exceptions of the whelk (number 14 on the diagram) and the curiously artificial bird (6) which was perhaps included simply to fill up a space. The whelk shown belongs to a species of *Murex* which was in classical times the raw

material of a great industry surrounding the extraction from its tissues of the dye Tyrian Purple. It does not make good eating, and its modern slang name in Naples (*sconcioglio*) is used in an expression of repugnance.

The animals are illustrated with faithful delicacy and accuracy. The only serious error detectable in the mosaic is in the depiction of the electric ray *Torpedo* (number 1 on the diagram), where nine ring-like mark-

ings are shown on the dorsal surface, although the number is nearly always five. As will be argued below, this represents an error of transcription on the part of the illustrator, and probably occurred between the fishmarket (where he must have made his preliminary sketches) and the execution of the mosaic *in situ*. The animals are portrayed in the freshly killed state; they were not observed from life in aquaria. This is evident in a number of cases where fins or other excrescences are illustrated in a collapsed state (as occurs in air but not in water).

No marine biologist could fail to appreciate and admire this extraordinary composition, and one wishes that present-day scientists and their illustrators were always so painstaking and accurate.

(1) Electric ray: *Torpedo torpedo*. This animal is characterised by its possession on the upper surface of several (usually five) dark false 'eyespots' (ring-like markings, not true eyes at all). It is remarkable that the artist has depicted nine such markings and it may be surmised that this represents an error of transcription from the artist's notes and sketches to the mural itself. He has mistakenly depicted the ray's real eyes and spiracles (Fig. 1) (exhalant openings for the respiratory stream) to be four extra false 'eyespots'. This is the only major inaccuracy detectable in the whole mural.

(2) Red mullet: *Mullus surmuletus*. This fish is tantalisingly damaged in the mural and the tendril-like jaw processes characteristic of *Mullus* are not shown, and other features of the snout can only be guessed at. Because of this damage, the possibility cannot be ruled out that this fish belongs to the related variety (or separate species, according to one authority) *Mullus barbatus*. Both occur in the Mediterranean Sea. They are among the most highly prized table-fish in the world, and are commonly cooked whole, not gutted. (Naples slang for these fishes is *fragaglie*.)

(3) Grey mullet: *Mugil* sp. A beautiful illustration of a beautiful fish. Grey mullets shoal in coastal waters where they especially favour areas of fresh-water inflow, and sometimes enter estuaries and lagoons. There are seven species in the Mediterranean Sea, and only an expert can distinguish them, using whole fresh fish. Highly esteemed for their flavour, grey mullets were in classical times thought to be the most gentle and holy fish because they were herbivorous.

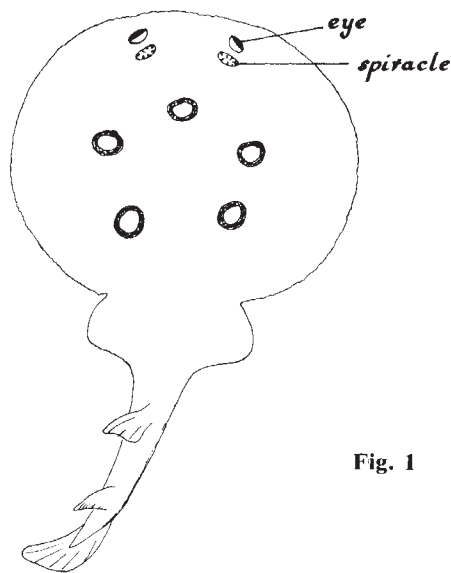


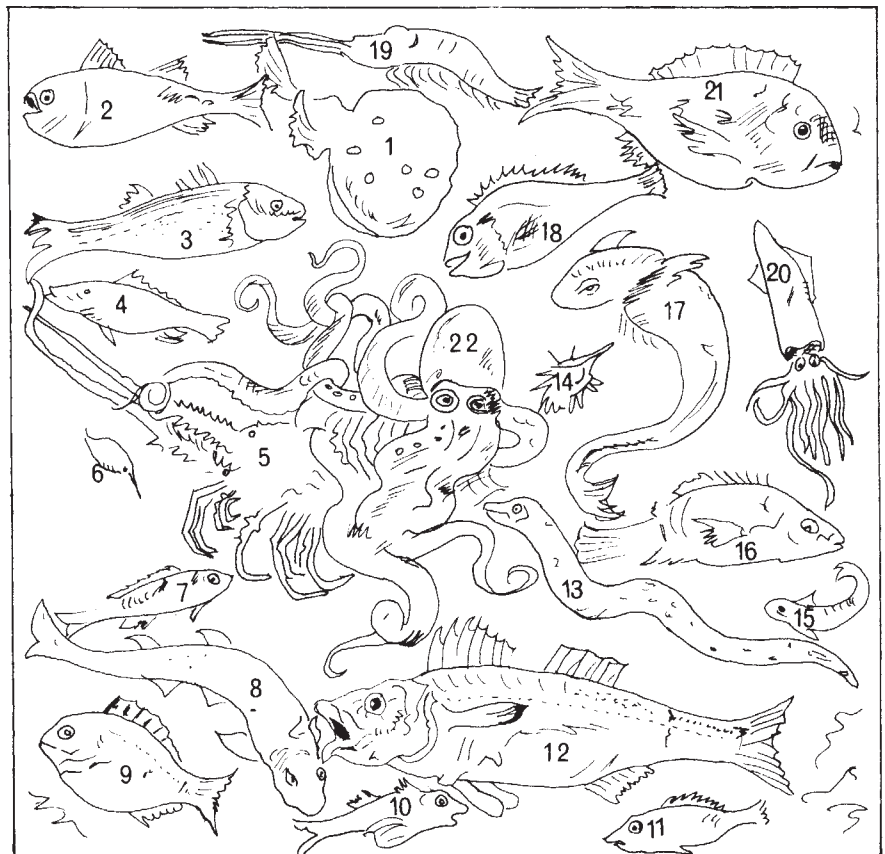
Fig. 1

(4) Wrasse: *Labrus* sp. This genus contains a number of well-established species but it is still difficult to identify the fish in the picture down to species. Wrasses include many common and familiar inhabitants of coastal waters in the eastern Atlantic, many of which are flamboyantly patterned. But the patterns vary with the time of the year and males and females are often quite different. Add to this difficulty the fact that many wrasses change sex as they grow up, and the reader will understand that it is difficult to be precise about this fish. *Labrus merula*, the brown wrasse, comes very close.

(5) Crawfish: *Palinurus vulgaris*, shown in combat (more likely to be imagined than real) with an octopus. It is interesting that the European crawfish is depicted and not the African or Green crawfish, which nowadays often finds its way to the fish markets of Italy. According to a cruel legend recounted by Robert Graves the Emperor Tiberius is said to have ordered a fisherman's death (the unfortunate man had startled the Emperor near the cliff-top in Capri) by the unusual means of having his own catch of crawfish (Suetonius writes instead that it was a mullet) rubbed into his face. He was then thrown from the cliff to the sea and rocks far below. Crawfish are nowadays best caught by SCUBA diving, but two thousand years ago they were probably trapped in baited wicker baskets.

(6) All scientists and illustrators are duffers outside their chosen areas of knowledge, and the author of the mosaic was evidently no ornithologist. This bird is a curious mixture of kingfisher, dunlin and rock pipit. It seems to have been included in the mosaic purely for artistic reasons. Whereas all the marine animals in the composition are represented faithfully and can usually be identified to genus and species, this is a generalised, stylised token bird, Disneyesque in its anonymity.

(7) Rainbow wrasse: *Coris julis*. This is a species which shows pronounced sexual dimorphism, the male



being very brightly marked while the female, presumed to be the fish depicted, is drabber. Such small fish were and are usually cooked whole, and form an essential part of the modern *Zuppa di Pesce*.

(8) Lesser spotted dogfish: *Scyliorhinus canicula*. This is the commonest small shark of the Mediterranean Sea and is taken by net or line on muddy and sandy ground in shallow waters. The flesh is unpleasant when fresh and fishermen often immerse the skinned bodies in seawater to achieve an acceptably flavoured fish (the taint is caused by the presence of a high concentration of urea in the fish's blood).

(9) Sea bream: *Diplodus sargus*. The artist has accurately depicted the dark markings near the tail and under the pectoral fin which distinguish this species from other sea breams.

(10) Red gurnard: *Aspitrigla cuculus*. This is a famous delicately flavoured gourmet fish. The artist has not illustrated the separate 'sensory' rays of the pectoral fin which are usually seen in the gurnards; perhaps they were collapsed or damaged in this specimen.

(11) This is probably a near relative of number 18, *q.v.*

(12) Sea bass: *Dicentrarchus labrax*. This excellent fish may occur in brackish lakes and estuaries as well as in the sea. According to Pliny, the Romans preferred specimens caught in the rivers ('*At in lupis, in amne capti praeferuntur*'), but things are not what they used to be, and nowadays those taken in the sea are better. Bass patrol rocky shores and pounce on shoals of sand-smelt or prawns. They will also maintain an uncanny watch on a SCUBA diver after the style of barracudas in the Caribbean.

(13) Moray eel: *Muraena helena*. This cunning and dangerous fish is highly esteemed by some gourmets, but others deem it worthy only of the *Zuppa di Pesce*. The skin is thick and slimy, but lacks scales. The artist has convincingly depicted the camouflage mottling and the gill opening just behind the mouth.

(14) This is in some ways the most interesting specimen in the mosaic, from the point of view of the marine biologist. It shows a neogastropod whelk having a long grooved siphon, the mark of a hunting snail. The length of this siphon proves the animal to be *Murex brandaris*, one of the snails used by the ancients in the manufacture of the dye Tyrian Purple, used for colouring imperial robes and other garments and furnishings. Whether the specimen illustrated was destined for the table or the dye factory is open to speculation; this species is, under the colloquial name of *murice* (in various parts of Italy) or *sconiglio* (around Naples) still eaten. The

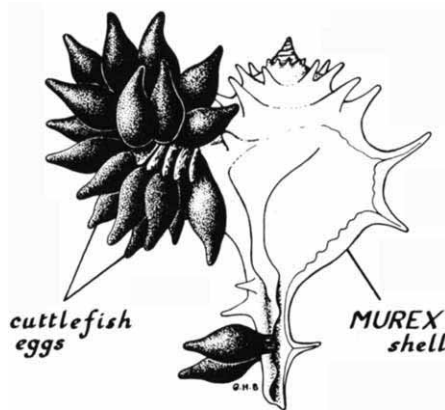


Fig. 2

modern Neapolitans use the expression 'ugly as a sconiglio' as an insult.

But this individual is especially interesting because it appears to show around the aperture of the shell a circle of flask-shaped excrescences. These hide the true spines of the shell, which are in any case slender and distinctive in this species, and it appears that they are the eggs of the cuttlefish *Sepia officinalis*. Such eggs are not infrequently found attached to *Murex brandaris* shells, and a drawing of such an association found on the island of Ischia (close to Naples) is reproduced here for comparison (Fig. 2). It seems that the artist may have selected for his sketches just such an individual, perhaps because the unadorned animal was even at that epoch regarded as ugly and uninteresting.

(15) A blenny, of which several species occur in the Mediterranean, in the genus *Blennius*. It is possible but not likely that it is instead a goby. Gobies were not thought highly of as table-fish, although a dissenting Venetian view was recorded by Martial:

'At Venice, famed for dainty dishes,

The Gobies rank the first of fishes.'

(from *Mediterranean Seafood*, Alan Davidson, Penguin Books, 1972)

(16) Striped bream: *Lithognathus mormyrus*. The tail of this species is usually found to be forked (as shown in the bream number 9) but the artist has here illustrated it in a collapsed state.

(17) Smooth hound: *Mustelus mustelus*. This small shark differs from the Mediterranean species of dogfishes (see number 8) by the forward position of the first dorsal fin. In the smooth hound this fin lies above the pectoral fins (more or less), while in the dogfishes it is more to the rear. The artist has shown this feature of shark classification extremely well.

(18) Scorpion fish: *Scorpaena porcus* or *S. scrofa*. There are ten species

of scorpion fish in the Mediterranean Sea, and it cannot be certain that the fish portrayed in the mosaic is one of the common ones. They are all bottom-living fish, hunting by twilight, seizing quite large prey with great speed, using the wide powerful mouth. So committed are they to life on the bottom of the sea that the scorpion fishes have lost the swim bladder characteristic of most other bony fish. The dorsal spines can inflict a painful wound if the fish is carelessly handled, and this fact, together with their generally small size, has meant that scorpion fish are utilised chiefly as a flamboyant ingredient in the Neapolitan *Zuppa di Pesce*. The fish in the mosaic was evidently sketched out of water, because the head processes (which normally stand up in water) are not illustrated.

(19) Shrimp: *Crangon vulgaris*. The artist has spread the peraeopods (walking legs) guilefully in order to illustrate all 18, but the chelae (nippers) are not shown so convincingly. This shrimp is usually found on muddy sand in shallow water, and it is drab, small and poor eating. One wonders why the artist chose to include *Crangon*, rather than the more exotic delicate-tasting 'scampi' (*Neophrops norvegicus*) or one of the larger prawns. Perhaps the gear to capture these was at that time non-existent.

(20) Squid: *Loligo vulgaris*. The diamond shape given to this squid by the position and extent of the gliding 'fins' serves to identify this species, sometimes called the long-finned squid. They usually swim very close to the surface of the sea and can be taken with bony fish in fishing nets. Typical specimens have eight short arms and two long ones, but the artist has shown one which lacks a short arm. Was this an inaccuracy or had this individual lost one?

(21) Two-handed bream: *Diplodus vulgaris*. The artist has shown the shape of a sea-bream well, but the species must remain a tentative identification. It is not easy to determine whether certain markings on the mosaic are merely accidental damage.

(22) Octopus: *Octopus vulgaris*. This is the 'Polpo' beloved of modern Italians. The artist has accurately depicted both the restless squirming energy of the octopus and the external features needed for an identification to species. The arms are shown to have two rows of suckers, typical of species of the genus *Octopus* (*Eledone* has single rows), and the proportions are of *O. vulgaris*, not the other Mediterranean octopus, *O. macropus*. Large specimens can reach 25 kg in weight, 3 m across the tentacles; not exactly a kraken, but pretty frightening to meet underwater. □

Restating the self-thinning rule

from Peter D. Moore

MORTALITY amongst young organisms is one of the basic observations which led Darwin to propound his theory of natural selection. Often this mortality results from the competitive stresses which are derived from a high density of other members of the same species. Among plants the process is often referred to as self thinning. The nature of this process was analysed by Yoda *et al.* (*J. Biol. Osaka City Univ.* **14**, 107; 1963) in pure even-aged populations of plants under density stress. Their most important finding was that if one plots the log of mean individual plant weight against the log of surviving plant density one obtains a straight line with a slope of $-3/2$.

The self-thinning rule which emerges from these observations can be summarised by the formula,

$$W = K\rho^{-3/2}$$

where W = mean plant weight and ρ = density.

The rule was examined by White and Harper (*J. Ecol.* **58**, 467; 1970), who provided new experimental data and who also analysed a body of published

data most of which agreed with the observations of Yoda *et al.* Variations in nutrient availability had no influence upon the slope of the line, but did affect its position on the axes. Fertility resulted in a higher degree of thinning. On examining published data concerning light intensity variations and thinning in *Helianthus annuus*, however, they found that decreasing light intensity was correlated with less steep slopes in the log plant weight/log density lines.

Kays and Harper (*J. Ecol.* **62**, 97; 1974) have since experimented with the rye grass, *Lolium perenne* and have tested the effect of light intensity upon both individual (genet) and tiller density and weight. At high light intensities genet weight and density obeyed the $3/2$ thinning law, but at the low light levels the slope fell to 1. On this basis they suggested that it is the reduced light intensity within a sward which causes the stress, resulting in mortality.

In this issue of *Nature* (page 330), Westoby re-examines the $3/2$ self-thinning law in the context of work on the influence of light on leaf mor-

phology. It is known that leaves produced under low light intensity have a larger area, but not normally a greater weight, than those produced in high light levels. Area: weight ratios are therefore raised at low light. This being so, Westoby has re-examined the original *Helianthus annuus* data analysed by White and Harper, but has plotted mean leaf area on a log scale rather than log mean plant weight. The result is a line with a $3/2$ slope which is independent of the light intensity at which the data was obtained. Thus he reformulates the self-thinning rule as

$$L = K\rho^{-3/2}$$

where L = leaf area. Not only is this formula of more general application than the original statement of the rule, it also permits one to predict mortality rates given information on the changes in leaf area index (leaf area: ground area) of a population in time. Such information is already available for many crop and forage species, hence one can work from this data to a better understanding of their population dynamics under density stress. □

Persistent infections without proviral DNA

from Robin Weiss

IN 1975, Zhdanov (*Nature* **256**, 471) and Simpson and Iinuma (*Proc. natn. Acad. Sci. U.S.A.* **72**, 3230) claimed to have found integrated DNA copies of RNA viruses such as sindbis, measles and respiratory syncytial viruses in the cells of persistently infected cultures. They suggested that the formation of DNA 'proviruses' might lead to the persistent state. Recently, John Holland and associates (Holland *et al.*, *J. gen. Virol.*, **33**, 193; 1976) have concluded from a series of meticulous experiments on long-term persistent cultures of BHK-21 cells infected with vesicular stomatitis virus (VSV) that there is no evidence for 'proviral' DNA copies of the VSV genome in those cultures. Hybridisation tests capable of detecting 1 viral genome per 40 cells (or 1/40 of a genome per cell) gave no indication of sequences homologous to viral RNA in the DNA of persistently infected cells, and DNA transfection attempts also failed to reveal any biological activity. Transfection with DNA was also negative in a variety of other cultures persistently infected with rabies,

influenza, measles, mumps or lymphocytic choriomeningitis viruses. Thus it is clear that the formation of proviral DNA is by no means a prerequisite for the establishment of persistent infections with negative strand RNA viruses.

Holland and Villarreal originally established the persistently infected VSV carrier culture (*Proc. natn. Acad. Sci. U.S.A.* **71**, 2956; 1974) by using a temperature-sensitive mutant (*tsG31*) together with an excess of defective interfering (DI) particles. Nucleocapsids of the DI particles persist in the carrier cultures and presumably interfere with complete particle replication. Nevertheless, most cells in the carrier cultures continue to synthesise viral RNA (Villarreal and Holland, *J. gen. Virol.* **33**, 213; 1976) and proteins even after more than 2 years in culture. Cell cloning can, however, yield 'cured' cell populations lacking all evidence of infection.

Holland's group's studies support the view put forward by Huang and Baltimore in 1970 (*Nature* **226**, 325-327) that defective interfering particles have

a role in viral disease processes. Doyle and Holland (*Proc. natn. Acad. Sci. U.S.A.* **70**, 2105; 1973) had previously shown that the addition of excess DI particles to biologically active VSV protected mice against early lethal intracerebral infection. But DI particles might lead to 'slow' persistent infection, as opposed to acute infection, *in vivo* too, and with some viruses (measles, for example) and some hosts (such as man) the consequences to the host may be far more dire than acute infection. Since DI particles depend on non-defective virus for replication, but then replicate to excess and interfere with the non-defective virus, cyclic production of mature and DI virus may ensue, as Palma and Huang (*J. infect. Dis.* **129**, 402; 1974) have shown for VSV when fresh cells are added to infected cultures. The mechanism of interference by DI particles is not clear, but they may compete for polymerase. DI particles typically contain a part of the viral genome as the usual negative strand RNA, although Lazzarini *et al.* (*J. molec. Biol.* **97**, 289; 1975) have described interesting covalently-linked

positive and negative RNA strands, that hybridise into double-stranded hair-pin forms.

Rabies is another rhabdovirus which, unlike VSV, spontaneously gives rise to persistent infections in cell culture without the addition of DI particles (Wiktor & Clark, *Infect. Immun.* **6**, 983; 1972). However, DI particles are soon found in the infected cultures (Crick & Brown, *J. gen. Virol.* **22**, 147; 1974) and Holland *et al.* show in their paper (*op. cit.*) that even after cloning, rabies virus quickly generates new DI particles. The rate of generation of defective virions may therefore determine whether a particular virus or strain gives rise to acute lytic or persistent infection.

Other virologists (Stanners & Goldberg, *J. gen. Virol.* **29**, 281; 1975; Youngner *et al.*, *J. Virol.* **19**, 90; 1976) have shown that temperature-sensitive VSV mutants can give rise to persistent infections and have emphasised the possible role of conditional mutants in viral disease. The champions of DI particles point out that TS viruses generate DI particles more readily, but a curious finding is that persistent infections initiated by wild type plus DI particles also generate a high frequency of TS particles among the mature progeny. Furthermore, VSV and many arboviruses replicate in insect cells without cytopathic effects and therefore the wild type readily establishes persistent infections in these cells (which are probably their ancestral as well as present hosts). Yet one finds, as Mudd *et al.* (*J. gen. Virol.* **20**, 341; 1973) have shown, that many of the progeny replicated in insect cells become temperature sensitive, and will not grow at 37 °C. Perhaps the virus strains that are virulent in warm-blooded animals represent heat-resistant variants rather than true 'wild types'. Clearly, there is plenty to think about and investigate in persistent viral infections and virus-host interactions without worrying about DNA copies.

Having returned to the question of unusual proviruses, however, it is noteworthy that Zhdanov's and Iinuma's papers indicate that reverse transcriptase was associated with the viruses in their persistent infections, and one wonders whether retroviruses and other viruses might interact genetically in mixed infections. Since many commonly used cell lines are persistently infected with C-type viruses (which can 'persist' as host genes), this question merits investigation. Incidentally, strange transcripts of retrovirus genes themselves have recently come to light. Retroviruses are classified as 'positive strand' RNA viruses as their genomic RNA has the same sense as mRNA. As they replicate by transcription of a

DNA provirus one would not expect to find negative strand viral RNA as a replicative intermediate. Recently, however, Stavnezer *et al.* (*J. Virol.* **20**, 343; 1976) have detected by hybridisation to virion RNA the presence of negative RNA strands in the nuclei and cytoplasm of infected cells, representing up to 30% of the genome in avian sarcoma virus infection, and 45% with murine mammary tumour virus. Previous attempts to detect negative strand RNA may have failed because the RNA is already duplexed with positive strands. The function, if any, of the negative strand RNA is not yet clear. □

Cosmic X- and γ -ray line radiation

from Andrew Fabian

X- AND γ -ray astronomers have been observing an increasing number of spectral lines over the past year or so. Most of the X-ray reports have come from two groups: the OSO 8 group at the Goddard Space Flight Center; and the Ariel V group at the Mullard Space Science Laboratory of University College London. Line radiation at about 7 keV, presumably due to iron (which is by far the most abundant element emitting at this energy), has been detected to date in one binary X-ray source, Cyg X-3; several supernova remnants (for example, Cas A, Tycho and G287.8-0.5) and several clusters of galaxies (the Perseus cluster for example). All of these results (reported in the *Astrophysical Journal* and the *Monthly Notices of the Royal Astronomical Society*) derive from satellite-borne proportional counters, which have good sensitivity but poor energy resolution. The few high resolution crystal spectrometers carried into orbit are not yet sensitive enough to resolve such features.

The mere detection of line radiation from clusters of galaxies strongly favours a thermal origin for the X-ray emission, and indicates that the hot gas responsible cannot be wholly primordial. Presumably some heavy element enrichment has taken place. Most of the heavy elements are generally considered to be made in supernova explosions, and X-ray spectroscopy of their remnants may offer a direct means of checking that hypothesis. The preliminary results indicate that iron need not be particularly overabundant, but that result is, of course, dependent upon the structure of the remnant, and the proportion of ejecta to swept up

material currently radiating X rays. Cyg X-3 is a particularly enigmatic binary source, for it emits throughout the spectrum from radio waves to γ rays. Indeed there is no real proof that it is a binary source, for any companion is only inferred indirectly from the observations. Whereas the iron line radiation from the clusters of galaxies and supernova remnants is probably due to transitions in Fe XXV and Fe XXVI, the feature in Cyg X-3 may be due to fluorescence of 'cold' iron. A slight improvement in sensitivity and resolution should decide this point, since the 'hot' and 'cold' features differ significantly in energy. Variations in intensity over the 4.8-h cycle of Cyg X-3, and any future detection of periodic Doppler shifts in the line energy will undoubtedly be important in sorting out the true nature of this system. This work is only in its infancy, and the detection and resolution of more X-ray lines due to iron and the lighter abundant elements will be a worthwhile task.

γ -ray lines observed at energies above 0.511 MeV (the electron-positron annihilation line) are presumably due to nuclear transitions. Such radiation has been predicted to occur due to radio activity in supernovae and the interaction of low energy cosmic rays with interstellar material, and has been observed in intense flares in the Sun. A group at Rice University, Texas, have recently published a detailed account (*Astr. J.* **210**, 631-641; 1976) of their earlier reported detection of nuclear γ -ray lines in Centaurus A. At ~ 5 Mpc, it is the nearest bright radio galaxy, and contains a nucleus varying at X- and soft γ -ray energies. R. D. Hall, C. A. Meegan, G. D. Walraven, F. T. Djuth and R. C. Haymes claim the detection of features at 1.6 MeV and 4.5 MeV, both 3.3σ above the neighbouring continuum. Since they have about 40 energy channels in their balloon-borne scintillation counter, and they could have seen lines in any of these, this result is significant only at the $\sim 2\%$ level. Similar results on the galactic centre region have earlier been reported by the same group. Centaurus A is a reasonable place in which to expect plenty of low energy cosmic rays, and the absorption observed at X-ray wavelengths indicates the presence of a considerable amount of interstellar gas. It will be exciting if the intensity of the γ -ray line activity is found to vary in a similar fashion (but out of phase?) to the X- and γ -ray continuum. The authors note that the total low-energy γ -ray luminosity of Centaurus A is about 10^{44} erg s $^{-1}$. Since this is about ten times its radio luminosity, perhaps we had all better start referring to it as the nearest γ -ray galaxy. □

Regulating gene expression in differentiation

from Carol K. Klukas

CONSIDERING the number of different cell types which exist in any mature eukaryotic organism, and the number of individual differentiation events which must have occurred in precisely ordered sequence in the course of its development one is left in no doubt that the system which so precisely controls genome expression in eukaryotes must be incredibly complex.

Research into many different aspects of the control system is currently in progress in many laboratories, and a recent paper from Strom and Dorfman (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3428; 1976) offers new evidence in support of the theory first put forward by Britten and Davidson, that the intermediate repetitive sequences in the genome may have a crucial role in regulating gene expression.

An early theory of the control of eukaryotic gene expression which proposed that appropriate structural genes are singled out and amplified during differentiation has generally proved to be untrue. Gene titration experiments have indicated only one or at most a few copies of any one structural gene, even in cell types where the protein that the gene specifies makes up a large proportion of the total protein synthesised. Research has therefore concentrated on the investigation of possible transcriptional controls, such as the interaction of non-histone proteins and hormones with chromatin, and the sequence organisation of the DNA itself. In 1969 Britten and Davidson (*Science* **165**, 349; 1969) first suggested how, theoretically, intermediate repetitive sequences might control gene expression, and more recently (Davidson *et al. Cell* **4**, 217; 1975) they have found that 80–100% of the mRNA molecules present in sea urchin embryos are transcribed from single copy DNA sequences which are adjacent to interspersed repetitive sequences in the genome. This finding does indeed suggest the regulatory importance of these sequences.

In a recent issue of *Proc. natn. Acad. Sci. U.S.A.* (**73**, 3428–3432; 1976) Strom and Dorfman offer new data which more directly supports this theory and which suggests a specific mechanism by which such intermediate repetitive sequences in the DNA may control differentiation. They use the chick limb bud system in which chick stage 24 limb bud mesenchymal cells are dissociated and cultured *in vitro* at high density. Once the cell

density becomes greater than confluency these cells differentiate into cartilage unless the thymidine analogue 5-bromo-2'-deoxyuridine (BUdR) is present at a concentration of 32 mM during the initial 48 h of culture in which case differentiation is irreversibly inhibited. In this study DNA was prepared from early cultures of these limb bud cells grown in ³H-BUdR. The reassociation rate of this DNA was then measured after shearing and in the presence of a 100-fold excess of DNA prepared from cells at various stages of cartilage differentiation or from liver tissue or from whole chick embryos. These experiments show that sternal cartilage DNA and 8-day cultured limb bud cartilage DNA drive the reassociation reaction about twice as fast as does liver DNA or undifferentiated limb bud DNA or DNA from 8-day limb bud cultures blocked from differentiation by BUdR treatment. Furthermore, by analysing the reassociation kinetics of these reactions Strom and Dorfman determine that the total percentage of the tritium which reannealed in the moderately repetitive fraction was lower in liver DNA (20%) than in sternal cartilage DNA (30%), 8-day limb bud culture DNA (30%), or in total embryonic DNA (33%). These data suggest that in cells differentiated for cartilage production a certain set of intermediately repeated sequences is amplified, whereas in undifferentiated cells or in cells differentiated for other functions, this particular complement of intermediate repetitive sequences is not amplified.

Does this mean that the determinative event in the differentiation of cartilage cells might be the amplification of cartilage-specific sequences initially present in multiple copies? Several facts about this system suggest this to be true. First it must be noted that during the first 48 h of culture during which the differentiation process can be blocked by the presence of BUdR, very little cell division occurs. After 48 h the differentiation process is no longer sensitive to BUdR. Since it is known that the half life of ³H-BUdR in substituted DNA is 11 times shorter than ³H-TdR, it stands to reason that the mechanism of BUdR inhibition of differentiation is that although the amplification of cartilage-specific intermediate repetitive DNA sequences does occur in these cultures during the first 48 h, the amplification is accompanied by and followed by rapid degradation of the sequences (because they contain BUdR) so that the cell does not receive the proper differentiation signal.

Similar BUdR sensitivity is exhibited by differentiation processes in various other chick cell types and in other tissues of diverse eukaryotic organisms.

Could it be that this proposed amplification event is a generalised feature of the cell differentiation process and that it is somehow involved in the regulation of genome expression? A positive answer to this question is certainly possible, but regardless of the outcome, further reports of research on this phenomenon are sure to be forthcoming soon and certainly promise to be exciting. □

Plasmids and pathogenicity

from David Sherratt

ESCHERICHIA COLI has often been considered a harmless inhabitant both of the large intestine of man and animals and of molecular biology laboratories. However in the past ten years it has become increasingly clear that some strains of *Escherichia coli* can be pathogenic. Enteropathogenic strains in the gut cause diarrhoeal diseases and invasive strains cause bacteraemia and urinary infections. Diarrhoeal disease resulting from enteropathogenic *E. coli* may well be one of the leading causes of death of young children in developing countries (see Sack, *A. Rev. Microbiol.* **39**, 333; 1975), and enteropathogenic *E. coli* cause considerable losses in intensively reared livestock.

Enteropathogenic *E. coli* strains produce either or both of two types of protein exotoxin, one heat stable (ST) the other heat labile (LT). The latter resembles cholera toxin and produces a similar but comparatively short-lived massive salt and water secretion from the mucosal cells of the small intestine—hence the diarrhoea. The similar effects of ST toxin are even more short-lived and the biochemical basis for its action at the cellular level is unknown. H. Williams Smith (*J. gen. Microbiol.* **52**, 319; 1968) was the first to show that toxin production in *E. coli* is transmissible by conjugation, and this led to the finding that the toxins are plasmid specified, often by the same plasmid.

Little is known about the genetic determinants of toxins in other microorganisms, partly because little is known of their molecular biology. The genes for cholera toxin seem to be chromosomally located and the structural genes for diphtheria toxin are known to be located on a lysogenic bacteriophage. In addition, the genes responsible for causing crown gall tumour in plants seem to reside on a plasmid present in the causal microorganism *Agrobacterium tumefaciens*.

In an attempt to understand better the molecular nature of toxin synthesis, *So et al.* have recently cloned the genetic determinant of ST toxin (*J. Bact.* **128**, 463; 1976). Using the well established techniques of *in vitro* DNA recombinant research, they were able to clone a 5.7 megadalton *EcoRI*-derived DNA fragment determining toxin production in plasmid pSC101 which carries a tetracycline resistance marker, and in plasmid RSF2124 which is an ampicillin-resistant *ColE1* derivative. This latter plasmid is present in 20–30 copies per cell and continues replication after chloramphenicol treatment of cells containing it, allowing isolation of large quantities of cloned DNA. Thus the tools are now available to investigate the properties of the toxin and its coding DNA sequence more thoroughly. The cloned sequence or a part thereof can be used as a 'probe' to detect rapidly the presence of ST toxin DNA and related sequences elsewhere. It should also now be possible to study transcription and translation of the cloned DNA, either in *E. coli* 'minicells' or *in vitro*.

This is the first published report of the cloning of a genetic determinant of bacterial pathogenicity, and it is interesting to note how these experiments conform to the various regulations and guidelines intended to control *in vitro* DNA recombinant research. In the USA, the NIH guidelines fairly explicitly categorise such experiments as requiring low containment (P2, EK1), since ST toxin is classified as of low pathogenicity. Such containment conditions at least would normally be used for handling the original toxin-producing strains. Experiments involving DNA of moderate pathogenicity, for example that isolated from *Vibrio cholerae*, would in addition require a disabled vector (P2, EK2), whereas DNA from organisms of high pathogenicity should not be cloned at present. In Britain, the situation is less clear, as experimental protocols and details of available facilities now have to be submitted for consideration by the Genetic Manipulation Advisory Group (GMAG), although the report of the working party on genetic manipulation indicates that cloning of DNA from "bacteria specifying toxins virulent to man" should only proceed with a disabled vector and under the highest physical containment (category IV), whereas cloning of "bacterial DNA non-pathogenic to man" may be carried out under minimal containment conditions.

Much then depends on whether ST toxin is considered virulent to man. It might be good practice for the newly constituted GMAG to consider and decide the categorisation for the *So*

et al. experiments! The authors of the paper discuss the possible hazards, implications and benefits of their work. They point out that the ST-toxin determinant was found naturally in *E. coli* as part of a common type of conjugative plasmid which often also contains drug resistance genes. Tetracycline resistance and ampicillin resistance, which are present in the cloning vectors, are often present in toxigenic *E. coli* strains. Therefore no new combinations of drug resistance and toxin production had been produced by the cloning experiments. In addition, the authors were aware that ST toxin production in *E. coli* K12 (the strain used in the laboratory) was unable to cause disease symptoms. Even production of both toxins is not by itself sufficient for a strain to be pathogenic. A host of other, as yet poorly-defined characteristics are required: some concern the properties of the bacterial cell surface and at least one, the K antigen, is plasmid encoded.

Taking this into account *So et al.* felt that the new toxin-producing plasmids, if properly contained, present less of a hazard than the original plasmid in its original strain. It is now known that many drug-resistance genes are readily transposable from replicon to replicon, allowing new combinations of plasmid-borne drug resistance to arise at high frequency. It seems likely that many other plasmid determinants including toxin production will also turn out to be transposable; therefore it looks as though any gene combination that can be made from *E. coli* plasmids by the techniques of *in vitro* DNA recombinant research can occur fairly readily in nature, and if suitable conditions (those provided by the indiscriminate use of antibiotics for example) are available, then hazardous combinations of drug resistance and genes for pathogenicity can be selected and may become a serious problem for the medical and veterinary profession.

Williams Smith has also shown (*J. gen. Microbiol.* **47**, 153; 1967; *J. gen. Microbiol.* **83**, 95; 1974) that production of α -haemolysin and a toxin known as Vir is plasmid specified and associated with pathogenicity of invasive *Escherichia coli* strains. Similarly the presence of plasmids determining colicin V production seems to enhance pathogenicity, apparently by increasing survival of bacteria in blood and the peritoneum. If this effect is the result of colicin V production, then one has the intriguing example of a colicin protein, which is normally only considered to be active in killing *E. coli*, being involved in pathogenicity.

Studies of bacterial toxins are of interest not only to the bacteriologist. Both cholera and diphtheria toxin

have already proved invaluable tools to molecular biologists studying eukaryotes because of their highly specific hormone-like interactions both with the cell surface and with their targets within the cell. Cholera toxin has been used as a tool to investigate membrane receptors, as a model for the study of hormone action and as a cyclic AMP probe (for example Finkelstein, *Microbiology-1975*, American Society for Microbiology, **237**, 1975). Similarly diphtheria toxin has been used to block translation of mRNA into protein by enzymatically depleting elongation factor 2 (EF-2) in eukaryote cells. There is also speculative material for the molecular evolutionist: are these toxins of eukaryote origin or can their interactions with the eukaryotic cell throw light on the relationships between eukaryotes and prokaryotes? □

Erratum

In the article 'Diamond platelets are interstitial carbon' (*News and Views* **265**, 208; 1976) line 5, paragraph 3 should read "... concentrations up to 1%" and not "up to 12%", and line 13, paragraph 5 (page 209) should read "No more than 3%" and not "No more than 5%".



A hundred years ago

THE electric light is becoming common in Paris in connection with works that have to be carried on during the night. A large lamp fed by a six-horse power has been established in the Avenue de l'Opéra, and others are employed in the Trocadero in connection with the building of the Exhibition Palace. The gramme machine and screw regulator are employed.

THE INTRA-MERCURIAL PLANET.—In M. Leverrier's last communication to the Paris Academy on the planet assumed to exist within the orbit of Mercury, it was mentioned that, with the elements adopted, or very similar ones, a solar conjunction would occur on March 22, and a transit over the sun's disc was possible, though uncertain. A close examination of the disc is therefore to be recommended on March 22 and 23, and there is reason to believe that observers in widely-differing longitudes are prepared to undertake it. If no transit should then occur, eight or nine years may elapse before one is possible at the spring node. From *Nature* **15**, January 25, 282, 285; 1877

review article

Molecular analysis of spontaneous somatic mutants

Kayode Adetugbo*, César Milstein & David S. Secher†

Eukaryotic structural gene mutations occurring spontaneously in a mouse myeloma cell line offer the opportunity to study somatic mutation in animal cells at the molecular level. Studies on the myeloma protein and on mRNA have enabled us to characterise four such mutants representing four different mutation mechanisms. The results may have some bearing on the origin of antibody diversity.

THE nature and rate of mutation in somatic cells is of considerable interest in connection with a wide range of biological phenomena including carcinogenesis¹, immunology² and ageing³. Many cases of somatic mutations in animals are known and several have been extensively studied at the phenotypic level (for example mosaicism of fleece type⁴). In no case, however, has the phenotypic variation been unequivocally shown to be due to a mutation in a given structural gene. In some cases, for example certain haemoglobinopathies⁵, non-inherited alterations of haemoglobin have been reported. But these could equally be new spontaneous mutants of the germ line cells. Studies of somatic variants in animals are limited by the death of the individual. For this reason most of the efforts to characterise somatic mutants in eukaryotes have been made on cells in culture. This was made possible by developments in cloning techniques⁶ and a considerable number of examples of clonal variants have now been reported^{7,8}.

It was originally suggested^{9,10} that variants in tissue culture were of epigenetic origin, that is heritable variations (as observed in differentiation) not involving alterations in DNA sequence. More recent reviewers, however^{7,11}, have concluded that many of the described variants are indeed genetic. But the localisation of these mutations to specific structural genes remains incomplete. The presence of altered proteins in variant clones provides preliminary evidence for structural gene mutations. Alterations in enzyme activity¹²⁻¹⁴, immunoreactivity^{13,15,16}, temperature sensitivity^{12,17-19} and electrophoretic properties²⁰ have been taken as evidence of structural gene mutations. Chemically-induced mutant cells with no hypoxanthine guanine phosphoribosyltransferase activity, for example, have been shown to contain material that cross reacts immunologically with the enzyme and is presumably inactive protein; this material comigrates with wild-type enzyme in SDS-polyacrylamide gels^{13,15}. Hochman *et al.*²¹ have purified the regulatory subunit of cyclic AMP-dependent protein kinase in mutants of mouse lymphoma cells; the mutant protein has altered temperature sensitivity and enzymatic properties. Such mutants are presumably due to structural changes in the protein and may well be the result of mutations in the structural gene. But these methods of analysis do not exclude changes in post-translational modifications such as carbohydrate addition, phosphorylation, processing of precursor molecules, and so on. These often modify physicochemical and biological properties of protein molecules.

Amino acid sequence analysis of the altered proteins, to allow a more exhaustive characterisation of mutants, is hampered by the difficulty in obtaining enough material. This can be, at least partially, overcome by the use of radioactive tracer techniques. This approach was successfully used for the preliminary characterisation of structural mutants of immunoglobulin genes in myeloma cells in culture^{16,22}. Further characterisation of such mutants, including complete amino acid sequence analysis, is possible. Preparation of large quantities of mutant protein is simple when the cells are grown as tumours in mice since the myeloma proteins accumulate in the serum.

Spontaneous structural gene mutants

The mouse plasmacytoma MOPC 21 is an experimentally-induced tumour which secretes an immunoglobulin (Ig) consisting of γ 1 heavy chains and kappa light chains [IgG1(κ)] (ref. 23). Both chains have been fully sequenced and the arrangement of disulphide bridges elucidated^{22,24,25}. The light (L) and heavy (H) chains are made up of pseudosubunits of ~ 110 residues, each containing a disulphide loop (Fig. 1). For further details of Ig structure and genetics see ref. 26). A line adapted to permanent growth in tissue culture has been derived and is known as P3 (ref. 27). This line has been repeatedly cloned and individual subclones have been screened for the presence of isoelectric focusing variants of the secreted immunoglobulin. The procedure (outlined in Fig. 2) allowed the analysis of a random sample of 7,000 subclones. Five mutant clones, secreting immunoglobulins of altered isoelectric points were detected, but one was lost. Figure 2 shows the other four, which we named IF1, IF2, IF3 and IF4. Initial sequence studies were at the radioactive level with material prepared in tissue culture²⁸. More conventional methods²⁹ required milligram quantities of material which was prepared by injecting the mutant cells into BALB/c mice. Tumours were generally obtained directly, but sometimes irradiated mice (400 rad) were used during the early passages. The yield of mutant proteins in the serum of the tumour-bearing animals was lower than in the wild type (Fig. 3).

A nonsense mutant

IF1 had a higher pI than the wild-type protein and its H chain a lower molecular weight than wild type by SDS-polyacrylamide gel electrophoresis (Fig. 3). Neuraminidase treatment, to remove specifically sialic acid, followed by isoelectric focusing, produced a further shift in IF1, but no effect on wild type³⁰. Thus, in addition to primary sequence differences (see below), IF1 Ig had sialic acid, whereas wild-type Ig did not. A comparison of the

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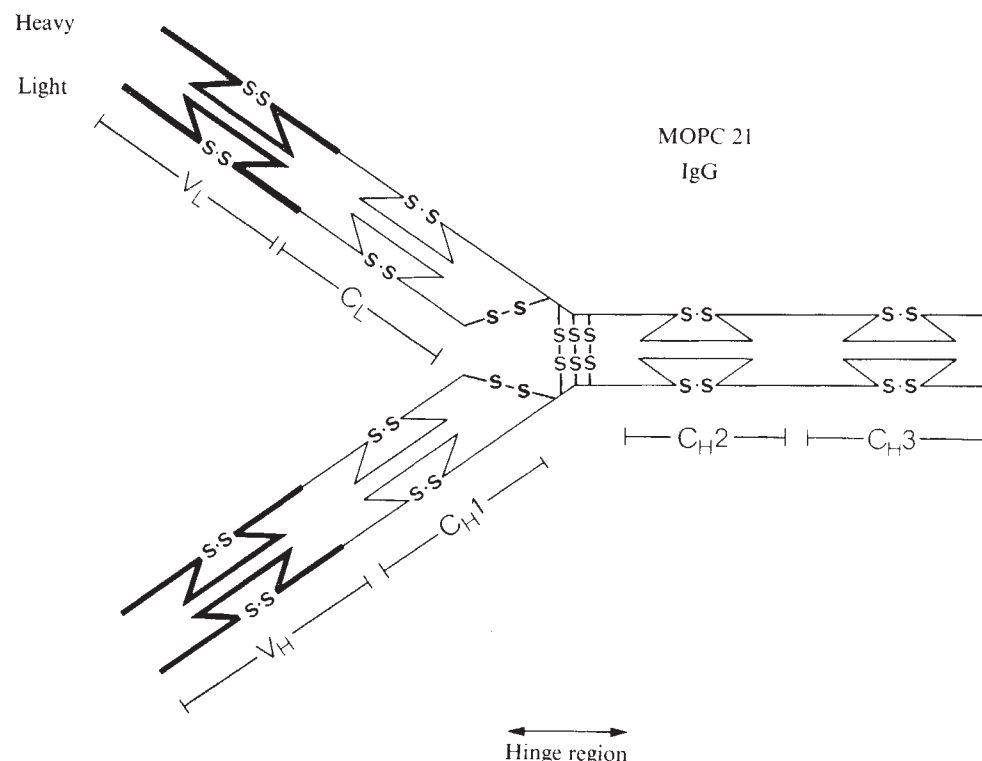


Fig. 1 Diagrammatic representation of the IgG molecule. The pseudo-subunits are named and the antigen binding domain (V region) is marked with heavy lines.

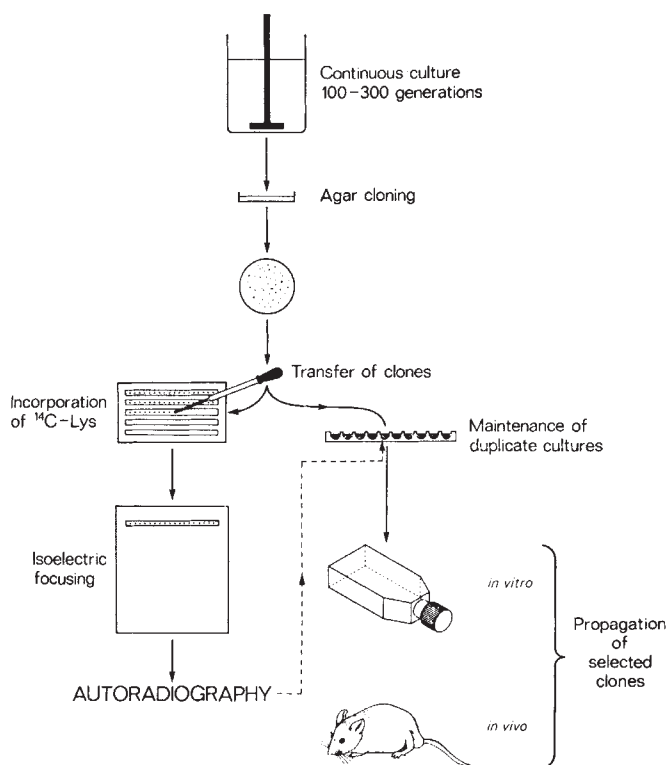


Fig. 2 Outline of the procedure used for the detection and isolation of mutant clones of P3. Cells were withdrawn from an aged (100–300 generations) spinner culture and subcloned in agar. When subclones were visible to the naked eye (12–14 d) they were transferred on to a layer of dialysis membrane on agar growth medium containing ^{14}C -lysine and incubated for 24 h. Replicas of sampled clones were maintained in microculture wells. After incubation the dialysis membrane containing the radiolabelled samples was applied on to thin slabs of polyacrylamide (4.5%) gels and isoelectric focusing was performed. The radioactive immunoglobulin bands were visualised by autoradiography. Further details have been published²⁰. Clones whose Ig bands show an altered isoelectric point were recovered from the replicas in microculture wells, grown to mass in suspension, and inoculated into BALB/c mice, in which they grew as solid tumours.

radioactive peptides from peptic and tryptic digestions of labelled wild-type and IF1 H chains²⁸ showed that the marker peptides from the $\text{C}_{\text{H}3}$ pseudosubunit (see Fig. 1) were missing. Cyanogen bromide fragmentation of IF1 H chain confirmed these findings. The three CNBr fragments of $\text{C}_{\text{H}3}$ (H8, H9, H10) (Fig. 4) were missing. A new CNBr peptide with N-terminal alanine was found, corresponding to the sequence Ala-Lys-Asp-Lys-Val₃₅₇ (ref. 29). Since this peptide had no homoserine, it was presumably the C-terminal fragment of IF1 H chain. The other CNBr fragments were identical to wild type fragments by amino acid analysis and peptide finger-printing techniques. No internal duplication of sequences was detected, so IF1H represents residues 1–357 of the wild-type sequence (Fig. 4), and is 83 residues shorter than the wild-type protein.

The mRNA from this mutant can be translated in cell-free systems. The translation product is smaller than the wild type, indicating that the mRNA itself is altered³¹. A comparison of the size of the mutant and wild-type H chain mRNA by sucrose gradient sedimentation showed that the protein deletion was not due to a corresponding deletion at the mRNA level. The mutation is therefore most probably due to a nonsense mutation in the codon of Ser-358 resulting in premature chain termination. The reason for the alteration in the carbohydrate composition remains unknown. It is possible that this is a secondary effect due to local changes in protein folding. This may lead to a new site for carbohydrate attachment or greater accessibility to sialic acid addition.

An internal deletion

IF2 IgG was more acidic than the wild type counterpart, and its heavy chain was smaller (Fig. 3). Neuraminidase treatment did not affect the $p\text{I}$ (ref. 30). Fingerprint studies of ^{35}S -cysteine and ^{14}C -lysine, ^{14}C -arginine labelled IF2 H chain showed that the $\text{C}_{\text{H}1}$ (see Fig. 1) peptides were missing and that the hinge region peptide was altered. CNBr digestion of IF2 H chain produced a new fragment, H56, in place of H5 and H6 (Fig. 3). A sequenator analysis of H56 gave the N terminal sequence:

108

Asp-Tyr-Trp-Gly-Gln-Gly-Thr-Ser-Val-Thr-Val-Ser-Ser-Val-Pro-Arg-Asp-CMC . . .

120 215

Thus, the 94 residues from 121 to 214 of the wild-type chain were missing from the sequence of this mutant and a new bond corresponding to Ser₁₂₀-Val₂₁₅ was found. The amino acid analysis of all CNBr fragments of IF2 H chain, the peptide maps and the amino acid compositions of the tryptic peptides²⁹, were consistent with the proposed internal deletion of 94 amino acid residues (Fig. 4).

The mRNA prepared from this mutant can be translated in cell-free systems to give a mutant heavy chain. Unlike IF1, the mRNA of IF2 does not comigrate in sucrose gradient sedimentation with the wild-type H-chain mRNA, but is significantly smaller. The decrease in size, from 17S to 16S, is consistent with an internal deletion of about 300 bases³¹. Since the mRNA is itself shorter, we conclude that the deletion originates in a deletion at the DNA level.

A frameshift mutant

IF3 was more basic than the wild type (Fig. 2), and its *pI* was not altered by neuraminidase treatment. As in IF1 and IF2, its L chain comigrated with wild-type L chain in SDS-polyacrylamide gels. The H chain had a faster mobility than (Fig. 2)

Ala-Pro-Gly-Val-His-His-Ser-Thr-Ser-Gln-Gly-Ala-Asp-Gly-Gln-Gly

It was concluded³² that this peptide corresponds to residues 339 to the C terminus, as the terminal glycine does not represent a point of tryptic, pseudotryptic, or CNBr cleavage. This peptide, plus those isolated for residues 304–338, completely account for the amino acid composition of IF3-H7'. Thus, the sequence of IF3 H chain is identical to the wild type in the first 340 positions. The sequence of the C-terminal 14 residues is not found in wild type, other mutants, or any other immunoglobulins, but it can be related to the sequence of wild type by postulating a -2 frameshift mechanism (Fig. 5). This explanation fits with the published mRNA sequence data and allows a unique sequence of 55 bases to be deduced for wild-type H-chain mRNA. It predicts that UAA ('ochre') is the terminator codon in the mutant. This is supported by experiments³³ showing that when IF3 H-chain mRNA was supplemented with UAA-suppressor tRNA in a cell-free system, suppression was detectable, and a new product larger than mutant but smaller than wild-type H-chain was found. No difference in the size of the mRNA was detected

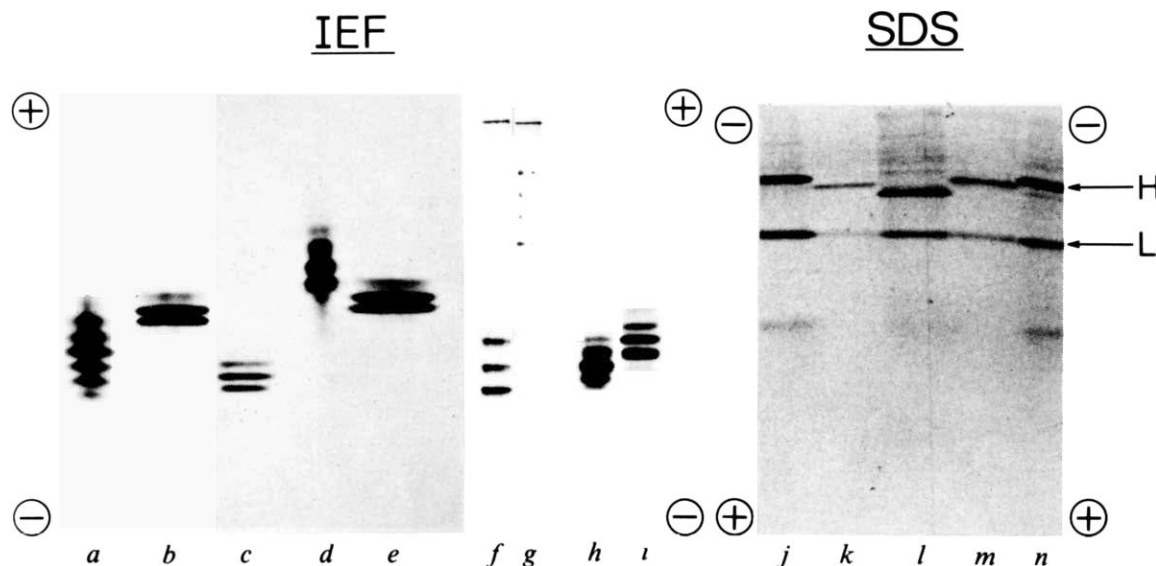


Fig. 3 Comparison of wild-type and mutant immunoglobulins (or Ig chains) by isoelectric focusing²⁰ (IEF) (a–i) or SDS-polyacrylamide slab gel electrophoresis⁴⁹ in a 20% acrylamide 0.065% bis-acrylamide gel (j–n). Igs were reduced by boiling with 2-mercaptoethanol in 1% SDS before applying to the gel. Ig was prepared either by the incorporation of ¹⁴C-lysine into secreted protein by cells in culture²⁰ (f, g, j–n), in which case proteins were visualised by autoradiography of the dried gel, or from the sera of tumour-bearing mice²⁵ (a–e, h, i). Typical yields of Ig ml⁻¹ of mouse serum were: wild type, 10–20 mg; IF1, 1–2 mg; IF2, 2–4 mg; IF3, 0.7–1.5 mg; IF4, 4–6 mg. For isoelectric focusing the pH gradients were: 5–8.5 (a–e), 6–8 (f, g), or 5–8.5 in 6 M urea (h, i). (b, e, f, n), wild type; (a, m), IF1; (c, k), IF3; (d, j), IF4; (g, l), IF2; (h), wild-type H chain; (i), IF4 H chain. +, anode; –, cathode. H, L, indicate the position of unlabelled marker wild-type H and L chain.

and a different amino acid composition from wild-type H chain²⁹. The three C-terminal CNBr fragments of the wild type (H8, H9 and H10, Fig. 4) were not present in IF3 H chain. The six N-terminal CNBr fragments (H1–H6) were unaltered, as judged by their amino acid compositions, the electrophoretic mobilities of fragments H4 and H5, and fingerprints and amino acid compositions of the tryptic peptides of the larger fragments. A new CNBr fragment, IF3-H7', was isolated²⁹. It had approximately the same size as wild-type H7, but a different amino acid composition. This CNBr fragment had no homoserine and was presumably the C-terminal fragment of IF3 H chain. Tryptic peptides corresponding to residues 304–338 from wild type were present in IF3-H7'. Fingerprinting of a tryptic digest of performic acid-oxidised IF3 H chain showed a peptide, IF3H-T28, that was not present in fingerprints of wild-type H chain. This peptide was isolated in very good yield and had the sequence:

by sucrose gradient centrifugation³¹. The mutant is therefore a frameshift mutant due to a deletion of two of the bases coding for Pro-Gln residues at positions 340–341 of the H chain.

A point mutant

IF4 Ig was more acidic than wild type (Fig. 3). The *pI* was not altered by neuraminidase. Isoelectric focusing of partially reduced and carboxymethylated chains showed that the difference was located in the H chain (Fig. 3). No differences were detected between wild-type and IF4 H-chains, by SDS-polyacrylamide gel electrophoresis or amino acid analysis.

The main product of limited peptic proteolysis of wild-type Ig is fragment (Fab')₂²⁵, a four-chain molecule made up to two intact light chains and two Fd' fragments (residues 1–232 of the H chain). The (Fab')₂ fragments from wild type and IF4 had identical *pI*, as did the main CNBr fragment (residues 143–303) of the H chains. The only detectable abnormality of IF4 (refs

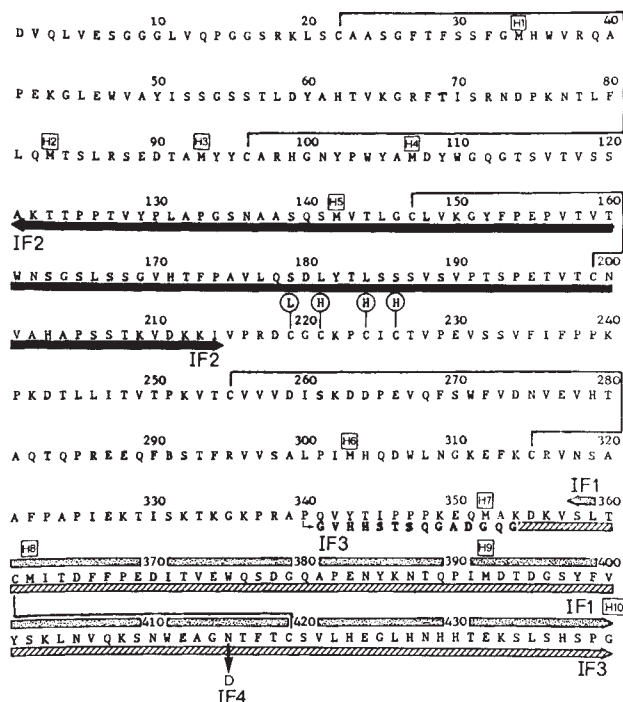


Fig. 4 The sequence of MOPC 21 H chain and of its mutants. The horizontal arrows indicate the extent of the deletion in each mutant. For IF3, an altered sequence continues for 14 residues beyond Pro-340 (see Fig. 5), and for IF4, Asn-415 is replaced by an Asp. H1, H2, and so on indicate the C-terminal residue of the respective CNBr fragments of MOPC 21 H chain.

29, 32) was the electrophoretic mobility (pH 6.5) of the ^{14}C -carboxymethylcysteinyl peptide H10T3 (ref. 34), residues 409–433 (Fig. 3) Thermolysin digestion of this peptide showed that while in wild-type Asx-415 was present in the neutral peptide Ala-Gly-Asn-Thr, in IF4 H chain it was in the acidic peptide Ala-Gly-Asp-Thr. The difference in structure between wild-type and mutant IF4 is therefore an asparagine-aspartate interchange at residue 415. (Pulse-labelled (15-, 30- and 60-min pulses) intracellular

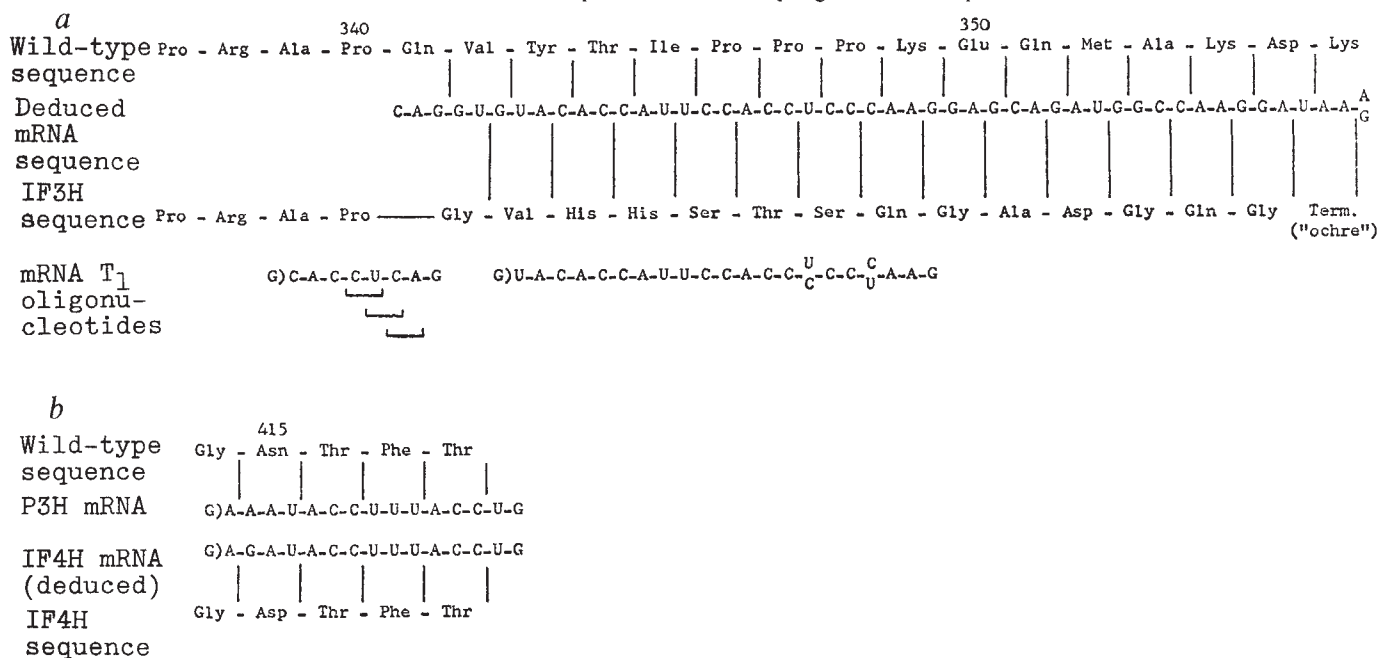
wild-type and IF4 immunoglobulins showed the same charge difference as the two proteins isolated from sera, indicating that the difference is not a result of deamidation. Further evidence on this point was obtained by fingerprint analysis of the H-chain mRNA from mutant cells. An oligonucleotide obtained by digestion with T_1 -ribonuclease (h6, Fig. 5), which was proposed to code for residues 414–419, had been previously isolated from ^{32}P -H-chain mRNA (ref. 31). The Asn $\rightarrow$ Asp interchange at 415 implies an A $\rightarrow$ G transition and therefore a split in oligonucleotide h6 at the new G residue. Fingerprints of IF4 ^{32}P H-chain mRNA showed that h6 was not present in the mutant. Unfortunately the predicted alternative oligonucleotide could not be positively identified. It is smaller than the wild type form and likely to be obscured by other oligonucleotides. The evidence indicates that the Asn-Asp interchange is the result of a point mutation.

Significance of the mutations

The mutations described above are not due to post-translational modifications. This was established by translation *in vitro* and structural studies of isolated mRNA. The possibilities that the mutant proteins are all due to post-transcriptional modifications of RNA or derived from genes silent in wild-type cells but expressed in the mutants, are not formally excluded but are most unlikely for several reasons. All the mutations are consistent with simple genetic events (Table 1) and no other primary sequence differences were detected. Each mutant represents a unique sequence, not observed in other myeloma proteins. Therefore, at least four, but probably more, such silent genes would have to be postulated. In addition, immunoglobulin V and C genes require an integration process for the expression of the mRNA^{26, 35}. This integration occurs during differentiation of stem cells into plasma cells^{36, 37}. The expression of a silent C gene would require a retranslocation of the V gene, since the V region of the mutants is the same as that of the wild type. Translocation of V genes onto new C genes in somatic cells *in vivo* gives rise to the expression of other known H-chain classes²⁶. This type of translocation has not been observed with plasma cells in culture. Attempts to detect them in established cell lines have been unsuccessful³². In other cases, however, it has been suggested that a modified C gene, with characteristics of another subclass, was detected in chemically-induced mutant cells as a result of deletion or unequal crossing over³⁸.

We conclude that IF1, IF2, IF3 and IF4 represent spontaneous

Fig. 5 The sequence of two stretches of wild-type heavy chain and of mutants. a, Sequence of the wild-type²² and IF3 H-chain can be read from a single mRNA sequence assuming a deletion of two bases, giving rise to a frameshift. Alternative positions of the deletion are indicated. b, Alteration of the point mutant IF4. T_1 oligonucleotide sequences taken from ref. 31.



mutations at the mouse γ 1 structural gene locus. They originate from four different mutation events (Table 1). Two of these seem to be point mutations—IF1 (a nonsense) and IF4 (a mis-sense). IF3 is a frameshift mutation resulting from a two-base deletion. The large deletion of IF2 may be due to a translocation or a mitotic recombination. All these processes have been well characterised in bacteria and lower eukaryotes. The studies described above show that they can take place in mammalian somatic cells. A statistical analysis of their relative frequencies is obviously premature. More examples may give some indication as to whether the greater complexity of the mammalian genome is associated with an alteration in the relative frequencies of the mutation events. In general, studies on mutations of somatic cells have involved mutagens. An important question is whether these agents merely increase mutation rates, or cause mutations that rarely (or never) occur spontaneously, or both. The studies above, although very restricted, provide a background on the more neglected area of

Variants of human kappa and lambda chains have been located in a homologous region. Residue 415 of MOPC 21 H chain, the O_2 marker of lambda chains²⁶, and the Inv marker of kappa chains²⁶ are, respectively, four, four and three residues before the second pseudosubunit half cystine. The mutations in IF1, IF2 and IF3 are all outside the pseudosubunit disulphide loops although more residues (247 residues) are within than outside (193 residues) the pseudosubunit disulphide loops (Fig. 4). This apparent bias in favour of mutations outside the loops could be the result of strong selective pressure at the protein level against mutations (or deletions) within the disulphide loops. In addition, normal mutation rates may be dependent on the DNA sequence^{42,43} and there might be mutational 'hot spots'. This is particularly applicable to the deletion of IF2 which starts at the V-C junction and ends at the residue repeatedly involved in deletions⁴¹. Selection might also occur at the mRNA level, against mutants with less than optimum base-pairing arrangements. Such mutants could lead to mRNA instability resulting

Table 1 Structural mutants of MOPC 21 (P3) mouse myeloma. The molecular weights are calculated from their sequences allowing 2000 daltons for carbohydrate

	Wild type MOPC 21	IF1	IF2	Mutants IF3	IF4
Molecular weight	50,800	41,300	40,900	40,700	50,800
H-chain defect		Deletion of C _H 3 (residues 358–440)	Deletion of C _H 1 (residues 121–214)	Altered sequence of residues 341–354 followed by deletion of C _H 3	Asparagine to aspartic acid (residue 415)
Sialic acid	—	+	—	—	—
Binding to F _c -receptor ⁴⁸	+	—	+	—	N.D.
H-chain mRNA size	17S	17S	16S	17S	17S
Proposed genetic mechanism		Point mutation (‘nonsense’)	Intracistronic deletion	Frameshift (–2) Premature ‘ochre’ termination	A to G transition (‘mis-sense’)

N.D. not determined.

spontaneous mutation. The procedures described above can equally be used to characterise the structural alterations induced by mutagens, particularly carcinogens.

Somatic mutation and antibody diversity

There were about 300 generations between the isolation of the P3 clone and the detection of mutants. Since five mutants were isolated from 7,000 subclones, the electrophoretic mutation rate of H-chain genes can be calculated, using the Catcheside approximation³⁹ as 3.3×10^{-6} per cell per generation. This mutation rate is higher than those reported for spontaneous auxotrophic mutants of Chinese hamster ovary cells in culture⁶ or for spontaneous variations in other mammalian cell systems^{12,17}, which are less than 10^{-8} per cell per generation.

From the H-chain sequence and the genetic code, we have calculated that 23% of all theoretically possible point mutations (two out of four in our case) will lead to a charge change²⁹. The corrected mutation rate (to include electrophoretically silent mutations) approaches 10^{-5} per cell per generation. This calculated rate must be a conservative estimate for several reasons. Certain mutations might lead to distortions in the protein structure causing problems of solubility and resulting in either a rapid catabolism⁴⁰ of the protein, or in cell death. In others, the mutant polypeptide chain might be too small to be detected by our methods. The low levels of mutant polypeptide chain might be too small to be detected by our methods. The low levels of mutant proteins found in the sera of tumour-bearing mice may be a result of any of these processes of selection.

The striking similarity between IF2 Ig and the human heavy-chain disease proteins⁴¹ has been stressed before²² and may be related to the mechanism of integration of V and C genes. The point mutation in IF4 also occurs in an interesting residue.

in the loss rather than the alteration of chains. The possibility that experimental immunoglobulin mutations may occur non-randomly along the chain has implications for the antibody diversity problem.

The functions of the immune system include the recognition of, and response to, an apparently limitless array of antigens. Variability in each Ig class or subclass is confined to the V regions, especially in the 50–70 residues that form the antigen-binding sites⁴⁴ in the V_H–V_L domain: the hypervariable residues. Recent results on quantification of the number of germ line genes by molecular hybridisation methods indicate that somatic mutation of antibody genes has a role in the diversification of Ig binding site sequences (reviewed in ref. 45). In the mutations reported here the V regions have not been involved. This may be due to the small number of mutants studied but it may be also that our cell line, which is already committed to Ig production, behaves differently from antigen-sensitive virgin cells. Cunningham and coworkers⁴⁶ have suggested that most combining site diversity occurs in a clone of cells after, and as a result of, antigen contact. If this is so, higher mutability should be expected on antigenic stimulation. However, the stability of a clone of antibody-producing cells *in vivo*⁴⁷ does not support such a view. Even so it is possible that uncommitted cells have a higher mutation frequency in their hypervariable region than more mature cells. We have suggested that some of the germ line genes include DNA sequences signalling higher mutation rates⁵⁰. This advantage may decline as mutants develop in the direction of more stable configurations.

The conservative mutation rate calculated here for the whole IgG is about 10^{-5} per cell per generation. Since hypervariable regions represent only 10% of the total molecule a mutation rate of 10^{-6} per cell per generation will affect the combining site. This means that even in committed cells a clone containing 10^6

cells expressing a single Ig may produce one new mutant in the combining site every generation—say 12 h. A population of 10^8 such cells (an average *Nature* reader has 10^{12} lymphoid cells) could thus generate 200 mutant genes per day. This number compares with current estimates of the total number of germ line antibody genes.

In summary, stable, spontaneous, somatic mutations have been detected in antibody genes in the absence of antigen or other avoidable selective pressure. The observed mutations have been apparently non-random. The mutation rate may be high enough to generate the diversity required from the germ line genes. Although our results do not directly implicate somatic mutation as a mechanism for antibody diversification *in vivo*, it seems likely that somatic mutation, coupled with antigen selection of new antibody combining sites, has an important biological role.

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articles

Mathematical models for the evolution of multigene families by unequal crossing over

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Mathematical models of homologous but unequal crossing-over between sister chromatids are presented. For mispairing by one repeat, the evolution of a multigene family by unequal crossing over can be represented by a linear birth-death process. The fixation rate of one repeat in a multigene family is estimated. For mispairing by more than one repeat, some approximate results are obtained.

EVIDENCE obtained during the past decade, reviewed in refs 1 and 2, has suggested that the eukaryotic genome contains multiple copies of certain nucleotide sequences, including genes. For example, satellite DNA isolated from *Drosophila virilis* contains about 10^7 copies of a heptanucleotide repeat known as satellite sequence I. Each cell also contains more than 10^6 copies of satellite sequences II and III, heptanucleotide repeats which could be derived from sequence I by single base substitutions. The gene

coding for 28S and 18S ribosomal RNA (rRNA) in various eukaryotes has been found to be randomly repeated². In the amphibian *Xenopus laevis* about 450 repeating units are clustered at the nucleolar organiser. Each unit has three major regions; 18S, 28S and spacer^{3–5}. The spacer regions, which are not transcribed⁶, are composed of a series of small tandem homology units. The number of these homology units differs from spacer to spacer and hence the spacers are heterogeneous in length^{8,9}. Except for the spacer regions the 450 ribosomal units of *X. laevis* are identical to one another within the accuracy of DNA–DNA hybridisation techniques^{4,7,10}. Comparison of the ribosomal repeats of *X. laevis* with its sister species *X. mulleri* shows that the 18S and 28S genes of the two species are indistinguishable, but the spacer regions differ widely in nucleotide sequence⁴. In *Drosophila melanogaster* tandemly repeated genes coding for rRNA (rDNA) are also clustered at the nucleolar organiser^{11,12}. There is one nucleolar organiser on each X and Y chromosome. A mutant known as *bobbed*¹² has been isolated that is partially deficient in rDNA genes, and hence, has

proven useful in the elucidation of a possible mechanism of gene magnification and reduction^{2,13}. Restriction enzyme analysis and nucleic acid hybridisation have shown that about 50% of the DNA in the human Y chromosome is a simple tandemly repeated DNA (ref. 14).

It has been suggested^{1,15} that sets of duplicated genes form multigene families, that is, are a group of nucleotide sequences or genes that exhibit the properties of multiplicity, close linkage, sequence homology and related or overlapping phenotypic functions. In particular, it has been argued that the genes that code for the variable regions of antibody molecules, V_L , V_K , and V_H , form three unlinked multigene families^{1,15-18}.

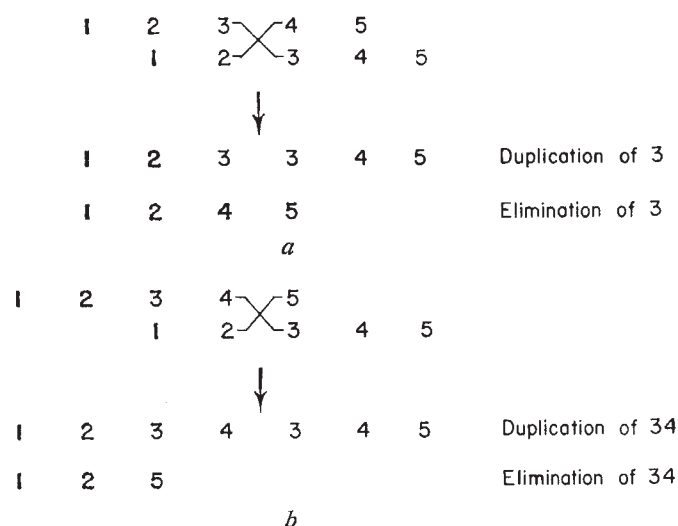


Fig. 1 Unequal crossing over between tandem sets of homologous genes and the products of the events. *a*, Mispairing by one gene; *b*, mispairing by two genes.

Genetic mechanisms need to be found which can not only generate repetitive gene sequences, but also explain the evolution of multigene families. Although the simplest hypothesis is to assume that the repeats in a multigene family evolve independently, there is evidence to the contrary. The genes within a multigene family can be maintained virtually identical within a species, and yet show divergence between species. This is the case with the spacer portions of the rRNA genes and the species-specific residues found in the conserved portion of the antibody V region that distinguish most immunoglobulins of one species from those of a second^{19,20}. This unusual evolutionary property of multigene families termed coincidental evolution¹, species-specific evolution¹⁹, horizontal evolution²¹ and co-evolution²², could be explained by parallel evolution, a gene correction process such as Callan's master slave hypothesis²³ or Gally and Edelman's democratic gene conversion theory²², or, alternatively, a process of repeated duplication of some genes in a family and a loss of others^{1,19}. Here we shall not discuss which of these proposals is most likely (for such discussion see refs 1 and 22), but we wish to point out that Tartof^{2,13,24} has evidence supporting the hypothesis that unequal mitotic sister chromatid exchange is responsible for the magnification and reduction of rDNA in *D. melanogaster*, and that some proponents of the germ line theory of antibody diversity have suggested unequal crossing over as a mechanism of formation for species-specific V region subgroups^{19,25-29}. Unequal crossing over has also been invoked to explain the origin of abnormal haemoglobulins such as haemoglobin Lepore³⁰ and haemoglobin Gun Hill^{31,32}, the variations in the length of the α chain of human haptoglobin³³, and the existence of the human γ -globulin hybrid γ G3- γ G1³⁴. Southern³⁵ suggested that unequal crossing over might also explain the long-range periodicities found in mouse satellite DNA.

Smith^{27,28}, Black and Gibson²⁹, and Tartof²⁴ have used Monte-Carlo computer simulations to study the evolution of multigene systems through unequal crossing over. They have found that after large numbers of crossovers, one gene in the multigene family tends to become fixed while all other genes are lost asymptotically.

Monte-Carlo simulations can only deal with limited numbers of genes. Thus there is no way to be certain that results obtained on multigene families with say 10^3 repeats hold for simple-sequence families which may contain as many as 10^7 repeats of a single nucleotide sequence. If unequal crossing over is to be a credible theory for the evolution of multigene families, large families need to be studied. Here we develop a simple mathematical model of sister chromatid crossing over which yields predictions consistent with the Monte-Carlo simulations and which allows the computation of fixation times for a family of any size.

Crossing over can occur either at meiosis or mitosis³⁶. Sister-chromatid exchanges have been observed to occur at frequencies of the order of 0.2 per chromosome per mitosis³⁷. During unequal, intrachromosomal crossing over, sister chromatids carrying closely linked homologous genes mispair, crossover and produce two new sister chromatids, one containing a greater and the other a lesser number of gene repeats (Fig. 1). A mispairing by one position before the crossover yields a duplication of a single gene in one chromatid and the elimination of the same gene in the other chromatid, while a mispairing by k positions leads to the duplication and elimination of k genes. Thus, in so far as the state of the genome is determined by the number of copies of each gene and does not depend on gene order, any state attained by unequal crossing over can be realised by a sequence of unequal crossover events where the mispairing is by one repeat.

Mathematical model

Assume that at time $t = 0$ there are N_0 adjacent homologous DNA sequences which represent a multigene family. These sequences need not be identical, only similar enough to allow mispairing. We also assume that of the N_0 sequences I are distinct sequences ($I \leq N_0$), called "genes". These distinct sequences need not represent a classical gene but rather may represent a repeat unit containing multiple genes as in the case of *Xenopus* rDNA. We shall follow one of these I genes, say gene i , through a sequence of crossover events and determine the probability, $P_{n_i}(t)$, of there being n_i copies of this gene at some later time t . We assume that at $t = 0$ each gene, including gene i , is present in a single copy so $I = N_0$. For $t \neq 0$, the total number of homologous sequences, $N(t)$, is a random variable which need not be equal to N_0 . Restricting our attention to mispairings by one repeat at any crossover events and determine the probability, $P_{n_i}(t)$, of there being n_i copies of this gene at some later time t . We assume that at one. If the probabilities of these events per unit time are $[1 - \lambda_{n_i}(t) - \mu_{n_i}(t)]$, $\lambda_{n_i}(t)$ respectively, a birth-death model³⁸ yields the following equations:

$$\frac{dP_{n_i}(t)}{dt} = -[\lambda_{n_i}(t) + \mu_{n_i}(t)]P_{n_i}(t) + \lambda_{n_i-1}(t)P_{n_i-1}(t) + \mu_{n_i+1}(t)P_{n_i+1}(t),$$

$$n_i \geq 1, \quad i = 1, 2, \dots, N_0 \quad (1a)$$

$$\frac{dP_0(t)}{dt} = -\lambda_0(t)P_0(t) + \mu_1(t)P_1(t),$$

$$n_i = 0, \quad i = 1, 2, \dots, N_0 \quad (1b)$$

with the initial conditions

$$P_{n_i}(0) = \begin{cases} 1 & \text{for } n_i = 1 \\ 0 & \text{for } n_i \neq 1 \end{cases}, \quad i = 1, 2, \dots, N_0 \quad (2)$$

The coefficients $\lambda_{n_i}(t)$ and $\mu_{n_i}(t)$ can be identified as follows. As crossovers occur the number and order of genes on the chromosome may change. If there is an equal *a priori* probability of each repeat being influenced by crossover (that is duplicated or eliminated), then the probability of a particular gene, say gene i , being influenced by a crossover at time t is $n_i/N(t)$, that is, its fractional occurrence. Further we shall assume that the probability that a gene influenced by a crossover is duplicated equals p and the probability of its being eliminated is $(1-p)$. (In the absence of any selective force one would expect $p=1/2$.) Hence, measuring time by the crossover rate (so that one crossover is expected per unit time),

$$\lambda_{n_i}(t) = P \frac{n_i}{N(t)} \quad (3a)$$

$$\mu_{n_i}(t) = (1-p) \frac{n_i}{N(t)} \quad (3b)$$

Notice that

$$N(t) = \sum_{i=1}^I n_i(t)$$

is a random variable. With the birth and death parameters given in equation (3), the probability of having n_i copies of gene i will depend on the number of copies of all the other genes. To proceed we shall replace $N(t)$ by its mean value, which for $p=1/2$ is N_0 . Thus we are approximating the birth and death rates λ and μ by their mean values. For other values of p , the mean value of $N(t)$ can be determined by considering a non-symmetrical random walk beginning at N_0 with an absorbing boundary at zero. In either event there is a finite probability for the loss of all the genes in the family; for $p \leq 1/2$ the ultimate loss is certain, while for $p > 1/2$ the extinction probability is

$$\left(\frac{1-p}{p}\right)^{N_0}$$

(ref. 38). When $p < 1/2$ the expected lifetime of the family, measured in crossover events, is $N_0(1-2p)^{-1}$ (ref. 38). For $p=1/2$ the expected lifetime is infinite, while for $p > 1/2$ the genes in the family may never be eliminated³⁸.

Under the assumptions $p=1/2$ and $N(t)=N_0$,

$$\lambda_{n_i} = \frac{n_i}{2N_0} = \mu_{n_i}, \quad n_i = 0, 1, \dots, N_0 \quad (4)$$

and equations (1) with initial conditions (2) reduce to the classical linear birth-death problem. The explicit solution, which may be found in Feller³⁸, is

$$P_0(t) = \frac{t}{t + 2N_0} \quad (5a)$$

$$P_{n_i}(t) = \frac{(t/2N_0)^{n_i-1}}{(1 + t/2N_0)^{n_i+1}}, \quad n_i \geq 1 \quad (5b)$$

Observe that as $t \rightarrow \infty$, $P_0 \rightarrow 1$ and $P_{n_i}(t) \rightarrow 0$, and hence all genes in the family will ultimately be lost. It is easy to verify that

$$\sum_{n_i=0}^{\infty} P_{n_i}(t) = 1$$

that the mean value of n_i ,

$$M_1(t) = \sum_{n_i=0}^{\infty} n_i P_{n_i}(t) = M_1(0) = 1$$

for all finite t , and that the variance $M_2(t) = t/N_0 + 1$. Hence for all

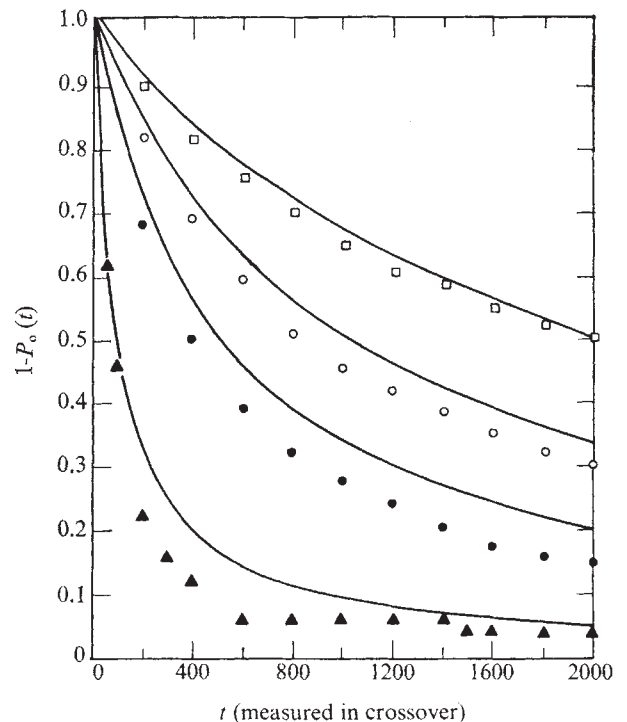
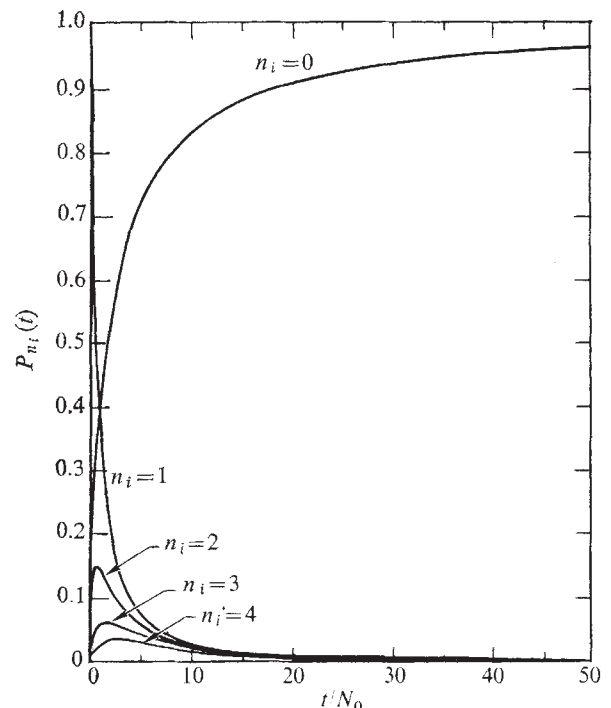


Fig. 2 Comparison of the predictions of the linear birth-death model (solid lines) with the Monte-Carlo simulations of Black and Gibson²⁹ (data points). The effect of the number of unequal crossovers and the size of the initial gene set on the rate of gene fixation. $\blacktriangle$, $N_0=50$; $\bullet$, $N_0=250$; $\circ$, $N_0=500$; $\square$, $N_0=1,000$.

finite times each of the original N_0 genes has a mean occurrence of one and a variance that increases linearly with time.

Even though the probability of any repeat being lost approaches one as $t \rightarrow \infty$, not all repeats will be lost in finite time, and in fact, one repeat will become fixed. One can understand this behaviour as follows: at $t=0$ there is one copy of each repeat. At the first

Fig. 3 The probability of having n_i copies of gene i at time t , measured in units of N_0 crossovers for n_i between 0 and 4.



crossover, with probability 1/2, one repeat will be eliminated, leaving no copies of it in the family. With the same probability some other repeat will be duplicated, leaving two copies of it. At a later stage in the process a repeat may have many copies, but there may still occur a streak of bad luck which leads to its elimination. Eventually, this will have occurred to all but one repeat. This repeat will then be fixed, and for $p = 1/2$, on the average, there will be N_0 copies of this repeat. As the process of unequal crossing over continues, the number of copies of this remaining repeat may also reach zero. In fact, the probability of this occurring will approach 1, but only as the number of crossovers approaches infinity. Since evolution only has had a finite time to proceed, this case is of no interest. Also, in reality, the loss of the genome would be highly selected against.

To assess the validity of replacing $N(t)$ by its mean value N_0 one can compare the results of Monte-Carlo simulations with our analytical results. Black and Gibson²⁹ performed such a simulation. They assumed that mispairing by one repeat occurs before each crossover, that $p = 1/2$, and they did not require that $N(t)$ remain constant. If one compares the Black and Gibson results for the percentage of the original gene types that remain in the family after a given number of crossovers with the predictions of the linear birth-death model one finds qualitative agreement (Fig. 2). The slight quantitative disagreement, especially at small values of N_0 , may stem from our setting $N(t) = N_0$, or may be due to poor statistics in the Monte-Carlo simulations which start with few repeats.

In Fig. 3 we plot $P_{n_i}(t)$ for n_i between 0 and 4. For $n_i > 1$ the maximum of P_{n_i} occurs at

$$t = (n_i - 1)N_0 \quad (6a)$$

and has the value

$$(P_{n_i})_{\max} = \left(\frac{2}{n_i - 1}\right)^2 \left(\frac{n_i - 1}{n_i + 1}\right)^{n_i + 1} \quad (6b)$$

The probability of finding exactly n_i copies of the i th repeat decreases after $t = (n_i - 1)N_0$ partially due to the further duplication of this repeat. The probability $P_{n \geq n_i}(t)$ of finding n_i or more copies of the repeat is, for $n_i \geq 1$, given by

$$P_{n \geq n_i}(t) = \frac{2N_0}{t} \left[\left(\frac{t}{2N_0 + t} \right)^{n_i} - \left(\frac{t}{2N_0 + t} \right)^{N_0 + 1} \right] \quad (7)$$

which for $N_0 \gg n_i$ is approximately given by

$$P_{n \geq n_i}(t) = \frac{2N_0}{t} \left(\frac{t}{2N_0 + t} \right)^{n_i} = \left(1 - \frac{t}{2N_0} \right) P_{n_i}(t) \quad (8)$$

Since all repeats are ultimately lost, $P_{n \geq n_i}(t) \rightarrow 0$ as $t \rightarrow \infty$, but goes through a maximum, for $N_0 \gg n_i$ at $t = 2N_0(n_i - 1)$. This is shown in Fig. 4.

Table 1 Probability of generating multiple copies

n_i	t/N_0	P_{n_i}	$P_{n \geq n_i}$
1	0	1	1
> 1	0	0	0
5	4	2.2×10^{-2}	6.6×10^{-2}
5	8	1.6×10^{-2}	8.2×10^{-2}
10	9	5.4×10^{-3}	3.0×10^{-2}
10	18	3.9×10^{-3}	3.9×10^{-2}
50	49	2.2×10^{-4}	5.5×10^{-3}
50	98	1.5×10^{-4}	7.4×10^{-3}
100	99	5.4×10^{-5}	2.7×10^{-3}
100	198	3.7×10^{-5}	3.7×10^{-3}
500	499	2.2×10^{-6}	5.4×10^{-4}
500	998	1.5×10^{-6}	7.4×10^{-4}

For some typical values of n_i , the number of repeats of the i th type, the probabilities $P_{n_i}(t)$ and $P_{n \geq n_i}(t)$ of finding exactly n_i copies and at least n_i copies, respectively, are given for $t = N_0(n_i - 1)$ and $t = 2N_0(n_i - 1)$. At these two times $P_{n_i}(t)$ and $P_{n \geq n_i}(t)$ attain their maximum values. In computing $P_{n \geq n_i}(t)$ we have assumed $N_0 \gg n_i$.

The probability of generating multiple copies of one particular gene by repeated unequal crossovers is surprisingly large, as illustrated in Table 1. Thus for $N_0 \gg 50$ in one out of every 135 organisms one gene would be replicated at least 50 times by unequal crossing over after $98N_0$ crossover events.

Gene fixation

Monte-Carlo simulations^{27,29} have shown that after a large number of crossovers one gene tends to expand at the expense of the other genes in the multigene family. When a given gene comprises a large fraction of the family, its probability of subsequent loss is small, and one says that the gene has been 'fixed'. One can estimate the gene fixation time in two ways: let $M_e(t)$ be the mean number of extinct genes at time t . Because $P_0(t)$ is the fraction of genes that are extinct,

$$M_e(t) = N_0 P_0(t) = \frac{N_0 t}{2N_0 + t} \quad (9)$$

If

$$M_e(t) = N_0 - 1 \quad (10)$$

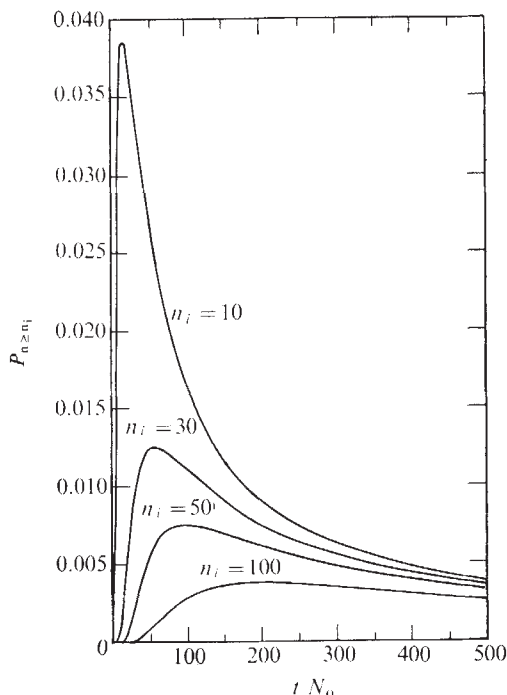
then the expected number of remaining genes is one. Combining equations (9) and (10) one finds that the expected fixation time, T_{bd} , determined by a birth-death process is

$$T_{bd} = 2N_0(N_0 - 1) \quad (11)$$

We have also computed the probability of one out of the original N_0 genes becoming fixed by time t , the probability of ultimate fixation, and show that the mean time to fixation in this birth and death model is precisely that given by equation (11). Our reasoning is as follows.

In the birth and death model we considered N_0 genes, each initially present in a single copy. The probability, $P_0(t)$, for any one of these genes to be extinct at time t was given by equation (5a). The

Fig. 4 The probability of having at least n_i copies of gene i at time t , measured in units of N_0 crossovers.



genes evolve independently in the model. Hence the probability $P_{N_0 \rightarrow 0}(t)$ that all are extinct at time t is

$$P_{N_0 \rightarrow 0}(t) = P^{N_0} = \left(\frac{t}{t + 2N_0} \right)^{N_0} \quad (12)$$

while the probability that only one survives, $P_{N_0 \rightarrow 1}(t)$, is

$$P_{N_0 \rightarrow 1}(t) = N_0(1 - P_0)P^{N_0-1} = \frac{2N_0^2 t^{N_0-1}}{(t + 2N_0)^{N_0}} \quad (13)$$

The rate of change of the sum of these two probabilities gives the rate at which the number of surviving genes is reduced to one, that is the rate of gene fixation,

$$\frac{dp_t(t)}{dt}$$

Thus

$$\frac{dp_t(t)}{dt} = \frac{dP_{N_0 \rightarrow 0}}{dt} + \frac{dP_{N_0 \rightarrow 1}}{dt} = \frac{4N_0^3(N_0 - 1)t^{N_0-2}}{(t + 2N_0)^{N_0+1}} \quad (14)$$

With this rate, the cumulative probability of gene fixation by time t , $P_t(t)$, is

$$P_t(t) = \int_0^t \frac{dp_t(t')}{dt'} dt' = \frac{(2N_0^2 + t)t^{N_0-1}}{(t + 2N_0)^{N_0}} \xrightarrow{t \rightarrow \infty} 1 \quad (15)$$

so that gene fixation always takes place and

$$\int_0^t \frac{dp_t(t')}{dt'} dt' = \frac{2N_0(N_0 - 1)t^{N_0}}{(t + 2N_0)^{N_0}} \xrightarrow{t \rightarrow \infty} 2N_0(N_0 - 1) \quad (16)$$

so that equation (11) gives the mean time to fixation in the birth and death model. Because one gene always becomes fixed, the probability of ultimate fixation of any particular gene is $1/N_0$.

Alternatively, Black and Gibson²⁹ measure the percentage of genes that become extinct. For example, from equation (9) the mean time it takes for 99% of the genes to become extinct is given by

$$0.99 = \frac{t}{2N_0 + t} \text{ or } t = 198N_0$$

If $N_0 = 100$, then one would expect that only one gene would remain after 19,800 crossovers. But since the mean value of $N(t)$ is N_0 , given that one gene remained, one would expect to find it present in 100 copies. As Fig. 3 shows, $P_0(t)$ rises rapidly, reaches 0.9 by $t = 18N_0$, and then slowly approaches its asymptotic value of 1.0. Thus this measure of the fixation time is very sensitive to the percentage criteria used when more than 90% extinction is specified. For example, while it takes 19,800 crossovers to attain 99% extinction of 100 genes, only 1,800 crossovers are required to reach 90% extinction. Further, if one wishes to determine the dependence of the fixation time on N_0 , a percentage fixation time is not suitable; for example, 99% elimination for $N_0 = 10^2$ and 10^6 leaves 1 and 10^4 remaining genes, respectively.

Estimates for the gene fixation time obtained from the birth-death model can be compared with Smith's²⁷ Monte-Carlo results. Smith lets $N(t)$ vary within set limits, chooses $p = 1/2$, and allows mispairing by a random number of positions as long as it is consistent with the prescribed latitude for $N(t)$. As a criterion for fixation he requires that the "clonality"

$$\left[\sum_{i=1}^{N_0} \left(\frac{n_i(t)}{N(t)} \right)^2 \right]^{1/2} = 0.9$$

This criterion, which is roughly equivalent to requiring that 95% of

the family be composed of copies of a single repeat, can be compared with requiring $n_i = 0.95 N_0$ in our model. Then the time at which $P_{0.95 N_0}$ is a maximum would logically correspond to Smith's fixation time. For the birth-death model this time, $t = (0.95 N_0 - 1)N_0$ given by equation (6a), will vary quadratically with N_0 for large N_0 . Smith found that fixation time increased approximately linearly with N_0 . Further, say for $N_0 = 500$, Smith finds fixation times of the order of 10^4 crossovers, whereas the linear birth death model predicts $t \sim 2.4 \times 10^6$. Equation (11) which requires a more stringent fixation condition predicts $T_{bd} \sim 5 \times 10^5$. These discrepancies with Smith's simulations are due to the fact that random mispairings by more than one repeat were allowed in the simulations.

Alternative models

In the model we have just considered, time has been measured in units of crossover events. If the number of crossovers within the multigene family per cell division were constant this approach would be appropriate. One can, however, envision the situation in which the number of crossovers that occur per unit time is proportional to the number of homologous genes in the family. Southern³⁵ has suggested that such a mechanism may account for the periodicities seen in mouse satellite DNA. Thus, suppose the rate of crossover influencing gene i is proportional to n_i so that $\lambda_{n_i} = k p n_i$ and $\mu_{n_i} = k(1 - p)n_i$, where k is the crossover rate per gene copy. For $p = 1/2$ this model is equivalent to the previous one with $N(t)$ replaced by k^{-1} . Thus it has the advantage of not requiring that $N(t)$ be approximated by its mean value, which for $p \neq 1/2$ would be time dependent. Hence, in this model, selective advantage (disadvantage) of a gene could be represented by using the known solutions to the linear birth-death problem³⁸ for $p > 1/2$ ($p < 1/2$).

A third model which leads to somewhat different results is to view the changes occurring at gene i as a gambler's ruin problem³⁸. That is, if we assume that there exists some biological mechanism for maintaining the length of the multigene family constant, say at N_0 , then gene i can contract until all copies are lost or it can expand until it attains length N_0 . We may again assume that there are equal probabilities for gene i expanding and contracting by one copy, that is $p = 1/2$. If gene i attains length N_0 , then all other genes are necessarily lost and gene i has been fixed. Since we have introduced no bias among the genes, the probability that gene i is fixed is $1/N_0$ and the probability that it is lost is $1 - 1/N_0$. When $p \neq 1/2$, as in a simulation of selective pressure, the probability of gene i being fixed is

$$\left(\frac{q}{p} - 1 \right) / \left[\left(\frac{q}{p} \right)^{N_0} - 1 \right]$$

where $q = 1 - p$ (ref. 38). As in the first model, one might suppose that the crossover probability per unit time for influencing genes i is n_i/N_0 . Using results on conditioned random walks developed in another context³⁸, we can estimate the mean time to gene fixation, T_{gr} , measured in crossover events and determined by a gambler's ruin process, as

$$T_{gr} = \begin{cases} (N_0 - 1)N_0 & p = 1/2 \\ \frac{N_0}{|q-p|} \ln \frac{N_0 + \sqrt{N_0^2 + 4S(S-1)}}{2S} & p \neq 1/2 \end{cases} \quad (17)$$

where $S = p/(p - q)$ for $p > 1/2$ and $S = q/(q - p)$ for $p < 1/2$. Smith personal communication obtained the same conclusion for $p = 1/2$ using Kimura's results on the fixation of mutant genes, and W. A. Beyer and M. S. Waterman (personal communication) have shown the result to be asymptotically valid as N_0 gets large. The corresponding time for the first model with $p = 1/2$, obtained by setting the clonality to 0.9 is $t = (0.95 N_0 - 1)N_0$, while the estimate obtained by setting $M_c(t) = N_0 - 1$ (see equation (11)) is precisely twice as long as that given by equation (17).

It is difficult to envision a biological process that would keep $N(t)$ constant. If the fitness of an organism is maximal when $N = N_0$

and decreases with the square of the deviation from this optimum amount, then, if mating is random and if recombination is equally likely to increase or decrease the genome length, Crow and Kimura⁴⁰ show that the chromosome length will be normally distributed about $N = N_0$. The gambler's ruin model should thus only be considered as an idealised model which gives another and independent estimate of the gene fixation time.

Multiple mispairing

To estimate the fixation time with multiple mispairing we shall use the diffusion equation method of population genetics⁴⁰. If x is the frequency of occurrence of a particular repeat, say the i th, we need to compute the mean $M_{\Delta x}$ and variance $V_{\Delta x}$ of the frequency change per unit time. We shall continue to assume that there is no selection so that $p = 1/2$. To maintain $N(t) = N_0$, however, we shall follow Ohta⁴¹ and assume that unequal crossovers occur in cycles composed of a duplication of m repeats followed by a deletion of m repeats, where the amount of mispairing per cycle, m , is a random variable. If the repeats of type i are distributed randomly among the N_0 repeats of the multigene family, then the probability of increasing the number of type i repeats by $\xi_1 N_0$ during a duplication event in which the mispairing is by m is given by the binomial distribution

$$P_{x \rightarrow x + \xi_1} = \binom{m}{\xi_1 N_0} x^{\xi_1 N_0} (1-x)^{m - \xi_1 N_0}, \quad 1 \leq \xi_1 \leq m \quad (18)$$

Analogously, the probability of then decreasing the number of type i repeats by $\xi_2 N_0$, given a mispairing by m , is

$$P_{x + \xi_1 \rightarrow x + \xi_1 - \xi_2} = \binom{m}{\xi_2 N_0} x^{\xi_2 N_0} (1-x)^{m - \xi_2 N_0}, \quad 0 \leq \xi_2 \leq m \quad (19)$$

where we have neglected the small changes in the gene frequency and repeat length caused by the preceding duplication event. Thus, for a series of cycles with random mispairings m occurring with frequency $f(m)$, and ξ_1 and ξ_2 taking on all possible values for a given m ,

$$V_{\Delta x} = \sum_{m=1}^{N_0} f(m) \sum_{\xi_1=0}^m \sum_{\xi_2=0}^m (\xi_1 - \xi_2)^2 P_{x \rightarrow x + \xi_1} \times P_{x + \xi_1 \rightarrow x + \xi_1 - \xi_2} \quad (20)$$

$$= \frac{2\bar{m}x(1-x)}{N_0^2} \quad (21)$$

where

$$\bar{m} = \sum_{m=1}^{N_0} mf(m)$$

Similarly, $M_{\Delta x} = 0$.

Using the results of Kimura and Ohta⁴² we then find that the mean fixation time measured in crossovers is

$$t_1 = \frac{-2N_0^2}{m} (N_0 - 1) \ln(1 - 1/N_0) \quad (22)$$

For unit mispairing the same results obtain with $\bar{m}$ replaced by one⁴¹. Thus the effect of multiple random mispairings is to decrease the fixation time by the mean mispairing $\bar{m}$.

Ohta⁴¹, using a similar diffusion equation analysis for the case of unit pairing unequal crossovers, computed $V_{\Delta x}$ and t_1 and obtained equations (21) and (22) with $\bar{m} = 1$. If we compare the diffusion equation results with the fixation time estimate derived by the birth-death process we find

$$t_1(\bar{m} = 1) = -N_0 \ln(1 - 1/N_0) T_{bd} \sim (1 + \frac{1}{2N_0} + \frac{1}{3N_0^2} + \dots) T_{bd} \quad (23)$$

where T_{bd} is given by equation (11). As N_0 becomes large the two estimates converge to the same result. For small values of N_0 , T_{bd} is probably a more accurate estimate since we do not need to assume that the repeat frequencies are continuous variables nor do we need to assume that duplication and deletion events strictly alternate (although we do assume that the average number of duplications and deletions are equal).

In Smith's²⁷ Monte-Carlo simulations $N(t)$ varied between $0.9 N_0$ and $1.1 N_0$. For $N_0 = 500$, mispairings between 1 and 50 occurred. If we assume that these occurred uniformly then $\bar{m} \approx 25$ and $t_1 \approx 2 \times 10^4$, which is in agreement with the Monte-Carlo results.

Future problems

We have constructed four mathematical models of unequal crossing over between sister chromatids. For the case in which there are equal probabilities of the offspring retaining the expanded or contracted chromosome (that is $p = 1/2$), we have given explicit solutions. The solutions have been interpreted to show that at long times one gene in a multigene family becomes fixed while the other genes are lost. Hence, as Smith²⁷ has pointed out, if the average time for a gene to acquire a mutation (the gene mutation time) is long compared with the time for gene fixation a very homogeneous multigene family would result which would seem to show coincidental evolution. On the other hand, if the gene mutation time was short compared with the gene fixation time a heterogeneous multigene family would be formed.

One might expect that selection would influence the relative probabilities of retaining the expanded or contracted chromosome. In *D. melanogaster* Tartof¹³ has found that even though expansion and contraction are concurrent events, offspring containing an expanded amount of rDNA are more prevalent than offspring containing a decreased rDNA content. It would be interesting to solve equations (1)–(3) or the related multi-dimensional random walk problem when $p \neq 1/2$ and $N(t) \neq \text{constant}$. Mathematically, one can view this unit mispairing problem as an urn problem in which N_0 balls each of a different colour are placed in a container at $t = 0$. A ball is chosen at random and replaced by two balls of the same colour as the chosen ball with probability p , and not replaced with probability $1 - p$. We present this problem as a challenge to mathematicians.

We have shown that, using a diffusion model, the time to gene fixation can be estimated for unequal crossing over in which the mispairing is a random variable. But we had to impose the rather stringent and biologically unreasonable assumption of the crossovers occurring in cycles of duplication and deletion events of identical length. Further, we assumed that the repeats were randomly arranged along the chromosome since correlations in the positions of initially adjoining genes will influence the probabilities of genes being duplicated or eliminated by unequal crossovers in which mispairing occurs by two or more repeats. To assess these correlations in analytical form and to relax the constraint that $N(t) = N_0$ seems to us to be an extremely challenging and difficult problem which we hope will attract some mathematical attention.

A realistic, but difficult mathematical problem is to enlarge the basic model so as to allow crossing over between homologous chromosomes in a diploid organism. At least in the case of *D. melanogaster* this does not seem to be necessary, for Tartof¹³ has found that unequal crossing over occurs predominately between chromatids on the same chromosome. Whether such restrictions also occur in other species where unequal crossing over has been adopted as an evolutionary mechanism remains to be seen.

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A fossil skull probably of the genus *Homo* from Sterkfontein, Transvaal

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A skull with numerous Homo features was discovered at Sterkfontein, near Krugersdorp, Transvaal, in August 1976. It stems from member 5 of the Sterkfontein Formation, with stone tools and with fauna pointing to an age of 2.0–1.5 Myr. The underlying member 4 contains Australopithecus africanus, no stone tools, and fauna dated 3.0–2.5 Myr. The new find supports the view that the Sterkfontein toolmaker was not the earlier A. africanus, but a later hominid related to Homo habilis.

By a remarkable double coincidence, the first pieces of the new skull, Stw 53, were found on August 9, 1976, 40 years to the day after Robert Broom's first visit to Sterkfontein, while the last part came to light on August 17, the 40th anniversary of Broom's first discovery of a hominid cranium at Sterkfontein. Although most of the fragments were found in a decalcified pocket of cave earth, one large part of the calvaria was still present in the calcified wall of the pocket, thus establishing indisputably the provenance of the specimen.

THE Sterkfontein cave deposit near Krugersdorp, Transvaal, has previously yielded a large number of fossil hominid bones that form the major part of the hypodigm of the species, *Australopithecus africanus*¹. Recently we extended our operations to a previously overburdened area south of the West Pit (formerly called the Extension Site). Here, between August 9 and 17, 1976 we brought to light a new fossil skull which, we believe, provides the first good evidence that the stone tools in the upper part of the Sterkfontein cave deposit were made by a hominid very similar to the early species of man originally described from Olduvai Gorge and designated *Homo habilis*².

The discovery was made 3 m south of the breccia from

which, in 1956–1958, Brain and Robinson³ had recovered the first stone tools from Sterkfontein. The breccia exposed in this part of the cave comprises Member 5 of the Sterkfontein Formation, according to Partridge. Member 5 contains a mammalian fauna that is much younger than that of the subjacent Member 4: according to Vrba's^{4,5} study of bovids in the two Members and other evidence, there may well be a lapse of ~0.5–1.0 Myr between the two Members. The long gap in the depositional sequence is the result of a marked unconformity between Members 4 and 5.

Apart from strong differences in bovid fauna, Members 4 and 5 differ in other respects: 4 contains no stone implements, while 5 contains an abundance of them. All of the hominid fossils unequivocally assigned to *A. africanus* emanate from 4, while until the new find was made, Member 5 had yielded only a handful of hominid teeth and jaw fragments. So unsatisfactory were these Member 5 remains that the identity of the hominid represented was uncertain. Robinson⁶ considered them to belong to *A. africanus*, like those from the deeper part of the cave, while Tobias⁷ regarded them as belonging to the genus *Homo* and probably to the species *H. habilis*. The new discovery provides the first unequivocal evidence of the nature of the Member 5 hominid and, probably, of the Sterkfontein stone tool-maker.

The new skull Stw 53

It is clear that we have a large part of the complete skull. We have the right brow, frontal bone and partially exposed frontal sinuses, several parietal fragments, including a posterior part of the left parietal bone articulating across the lambdoid suture with part of the occipital. The occipital includes a portion of the planum occipitale as well as of the planum nuchale, with a small, sharp nuchal crest on the medial, but not the lateral part of the bone. On the side of the cranium, we have a temporal, with well-preserved mastoid process, petrous pyramid and part of the tympanic

bounding the external acoustic meatus and ensheathing a bony styloid process which is still in place. Portions of the base of the cranium are available, including the lateral occipital with occipital condyle and the posterior part of the basi-occipital; portion of the floor and anterior wall of the middle cranial fossa, including the root of the lesser wing of the sphenoid bone. One lateral pterygoid lamina has been identified.

Of the face, there are present both nasal bones, complete from nasion to rhinion, supported by the left and right frontal processes of the maxilla. The pyriform aperture is virtually complete, including part of the floor of the nasal cavity. Most parts of the two maxillary bones are available, including the subnasal clivus, the palate and the alveolar processes. We have a large portion of the palate; the two maxillae have disarticulated neatly in the middle line. All of the front tooth sockets are present, though none of the front teeth has yet been found. On the right, all five cheek-teeth from P^3 – M^3 are available, with superbly preserved crowns and intact root systems. On the left, four of the five cheek-teeth are present; only the M^1 is lacking.

The mandible is thus far represented by only a good piece of the right ramus and possibly one or two additional fragments.

Morphology of calvaria

Salient features of the cranial and dental morphology are as follows. The skull as a whole is very small, though the curvature of the vault bones in the temporal fossa is full and rounded, quite unlike the marked constriction of this region found in the australopithecines, for example, Sts 5.

It is impossible at this stage to give an estimate of the cranial capacity, but we are confident that enough of the vault is preserved to make a reasonable estimate possible later. Two indications suggest a capacity that is moderately large for so small an individual: first, the well-filled vault in the temporal fossa and, secondly, the disposition of the temporal lines. These lines are modestly developed and they are well separated from the midline, both anteriorly on the frontal bone and posteriorly on the left parietal bone. The morphology of these temporal lines strongly suggests the pattern in *H. habilis* of East Africa.

The brow is very thin and moderately protuberant; in its delicacy and slight vertical thickness, it is reminiscent of Olduvai hominids 16 and 24, two of the specimens that have been referred to *H. habilis*.

The nose

The nasal bones are set at 180° to each other, except superiorly, where they have a slight linear elevation in the midline just below nasion. The well-preserved floor of the

Fig. 1 Maxilla of the new Sterkfontein skull, Stw 53, compared with SK 80, a Swartkrans maxilla of *Homo* species, originally called 'Telanthropus III'.

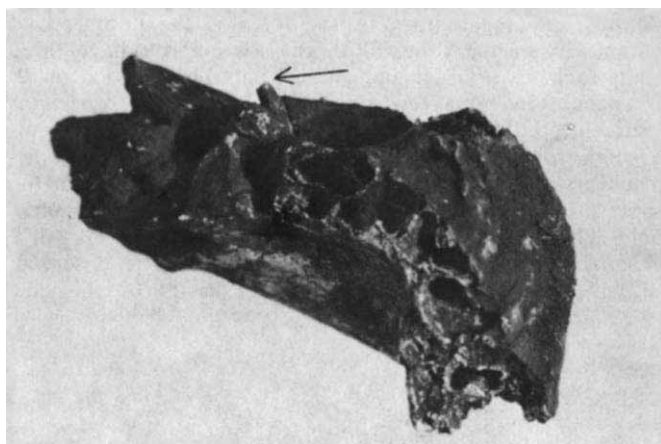
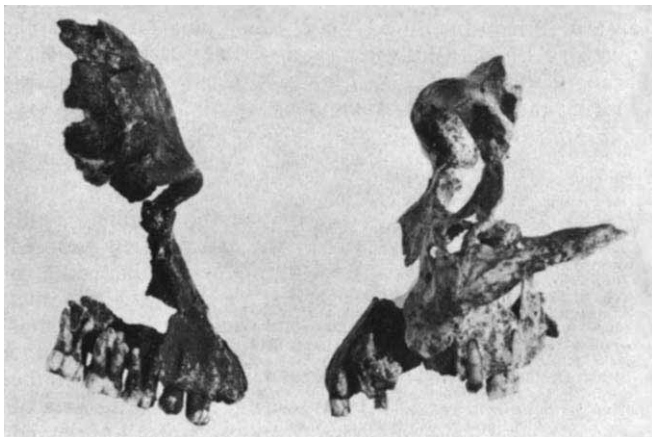


Fig. 2 Base of temporal bone of Stw 53 showing the ossified styloid process (arrowed).

nose, anterior nasal spine, nasal sill and subnasal maxilla are virtually identical to the maxilla from Swartkrans (SK 80) that Robinson⁸ recognised as 'Telanthropus III'. It was SK 80 that R. J. Clarke later found to join perfectly with cranium SK 847 and which he and Howell⁹ described as belonging to a species of *Homo*. The criteria that Robinson⁸ enunciated to distinguish between this area in *Homo*, on the one hand, and *Australopithecus* (both *A. africanus* and *A. robustus*), on the other hand, are still valid and they clearly distinguish the new Stw 53 maxilla from that of *A. africanus* and align it with *Homo*.

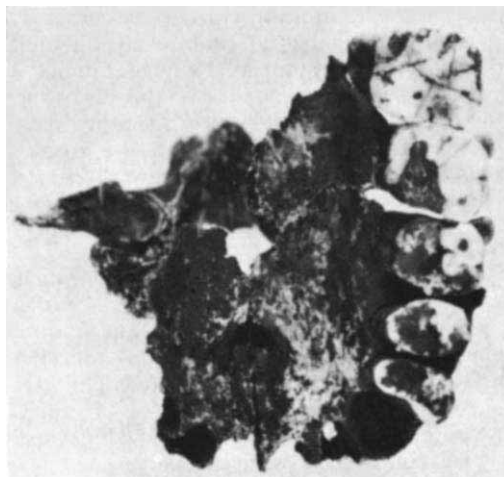
Cranial base

On the base of the cranium, the presence of an ossified styloid process is a feature that distinguishes Stw 53 from all previously described specimens of *Australopithecus* (Fig. 2). The lateral pterygoid plate, as R. J. Clarke pointed out to us, has a nearly vertical, notched posterior margin, like that of *Homo* and unlike the full, posteriorly-expanded, oblique posterior margin, in both robust and gracile australopithecines.

The teeth

The teeth are heavily worn, especially P^3 , P^4 , M^1 and M^2 , while there is moderate attrition even on the M^3 (Fig. 3).

Fig. 3 Palatal view of right maxilla with all five cheek teeth from P^3 to M^3 in position five.



A most striking feature of the teeth is the number and complexity of the roots. Both the P³s and P⁴s have three roots each, two buccal and one lingual. The position on P³ is encountered in a few of the Sterkfontein *A. africanus* teeth: according to Sperber¹⁰, of 7 Sterkfontein P³s, 1 has a single root, 3 possess two roots and 2 have three roots. Thus, in comparison with the Member 4 sample from the same site, the roots of the Stw 53 premolars are unremarkable. The position is, however, different with the P⁴. All 8 *A. africanus* P⁴s, the roots of which could be observed, display two roots¹⁰. In this respect, the P⁴ of the new

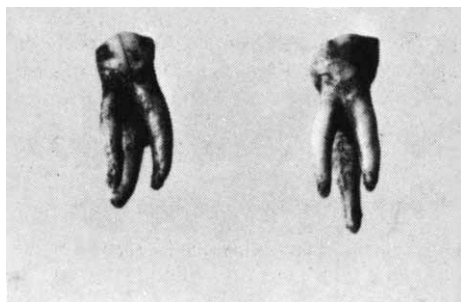


Fig. 4 Upper third and fourth premolars (isolated) to show the three roots of each.

Member 5 skull distinguishes itself from all specimens thus far known from Member 4 (Fig. 4).

It is interesting to note that in the Swartkrans maxilla (SK 80), which the Stw 53 so strongly resembles, Robinson states, "... it is quite clear from the sockets on the right side that P³ had three separate roots, the mesio-buccal one being closely approximated to the canine root"⁸. Unfortunately, only a part of the root and crown of "what is probably the right P⁴" is present and he did not refer to the root pattern reflected in the sockets for the P⁴ roots.

Three-rooted premolars are common in the Swartkrans fossils attributed to *A. robustus*. Thus, 8 out of 14 P³s and all but one of the P⁴s, from Swartkrans have three roots¹⁰.

There is a highly distinctive and florid development of the root system in the three molars. Each is furnished with two buccal roots and a sturdy lingual root, but each of these three roots is itself manifestly double and, at the tips, partially bifid. The duality of each of these three roots is more striking in M² than in M¹ and the degree of separation of each pair of two components is more marked. This is especially true of the mesio-buccal root, whose lingual component is so sundered from the buccal component as to place it virtually in the bucco-lingual midline of the tooth; its appearance is abnormal and this lingual rootlet curves strongly bucco-distad. The graded series apparent from M¹ to M² is continued into M³, where the process of root doubling of the mesio-buccal root reaches a climax with the marked distal recurvature of its lingual moiety (Fig. 5). In contrast M³ shows less duality of the disto-buccal root and of the lingual root. This pattern is sharply distinct from those of the australopithecines: in the latter group, scarcely a single one of the upper molars shows even a trace of grooves on the roots, let alone a frank doubling of each!

The mandible

The mandibular ramus is slender and gracile. The sculpturing on its medial face is clear cut and there is an acute angle between the endocoronoid and endocondyloid crests. The mandibular fossa is moderately developed as a flange in front of the endocoronoid ridge. The mandibular notch is somewhat excavated and it is fronted by an acuminate coronoid process. There is a distinct lingula. The features bear comparison with the corresponding part of Olduvai hominid 13 ('Cinderella'); but in the latter, the

sculpturing is accentuated and there is not so distinct a lingula. The part as a whole bespeaks a high, narrow, modern-looking ramus of the lower jaw.

All these features add up to a skull that seems to have a preponderance of individual features aligning it with early *Homo* species. It clearly has very little in common with *H. erectus* and would seem to be far too small-brained, thin-skulled and mildly crested to be accommodated in that taxon. On the other hand, the total picture indicates a strong affinity with the small-skulled, moderately-brained and structurally distinctive *H. habilis*. Stw 53 differs from *A. africanus* in much the same way as does the roughly contemporary, pygmoid, brainy *H. habilis* of East Africa, now known from Olduvai (1.8 Myr), Omo (1.85 Myr)¹¹ and East of Lake Turkana (?2.0–1.8 Myr)¹².

Conclusions

In this new discovery, we believe we have for the first time, convincing evidence that the Sterkfontein tool-maker belonged to the genus *Homo* and either to *H. habilis* or to a form closely related (*H. aff. habilis*). Furthermore, the positions of *A. africanus* in Member 4 and of *Homo* and stone tools in Member 5 strengthen the long-established hypothesis that *A. africanus* was earlier in time than early *Homo* (such as *H. habilis*).



Fig. 5 Mesial views of M¹, M², M³ to show the increasing degree of separation, from M¹ to M³, of the mesio-buccal root into two rootlets. The lingual component of the mesio-buccal root becomes progressively closer to the bucco-lingual midline of the tooth and is strongly recurved buccally and distally.

This view has gained important support from the newest evidence from East Africa^{11,12}. Moreover, the relative ages of *A. africanus* and *H. aff. habilis*, as revealed at Sterkfontein, supplemented by the evidence from East Africa are in keeping with the widely-held and strongly supported view that *A. africanus* is still the most likely claimant to have been ancestral to *H. habilis* and to later species of man.

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letters to nature

On the optical observation of galaxies with large redshifts

It is well known that the energy distributions of spiral galaxies rise sharply in the ultraviolet. Recent observations of six Sb and Sc galaxies¹ made with the TD1 satellite place this steep rise at considerably longer wavelengths than the earlier observations made with the OAO-2 satellite^{2,3}. If the TD1 results are correct, the large ultraviolet flux, which is now considerably closer to the U band, suggests the possibility of detecting more distant galaxies with deep ultraviolet plates than the usual deep B, V or R plates, since this region of high flux will be redshifted into the U band.

The problem of whether distant galaxies fall within the limiting apparent magnitude of a plate can be examined from the published energy distributions. Figure 1 shows the absolute energy distribution adopted for an Sb/Sc galaxy. Below 2740 Å the fluxes represent the average of the six galaxies measured by the TD1 satellite, the various intrinsic luminosities being allowed for by using published *V* magnitudes⁴ and normalising to a constant *V* magnitude. Above 2700 Å the relative fluxes taken from Pence³ were made absolute by using the flux for M31 (ref. 5) scaled to the chosen *V* magnitude; Pence's results below 3500 Å are, however, an extrapolation. It is encouraging to note the continuity between the two portions of the energy distribution, considering the large errors which may be involved.

Now the apparent magnitude, *m*, of a galaxy with absolute magnitude *M* is simply

$$m = M + 25 + 5 \log_{10}(d_L) + K_1(z) + K_2(z) \quad (1)$$

where d_L is the luminosity distance in Mpc given by⁶

$$d_L = \frac{c}{H_0 q_0} (q_0 z + (q_0 - 1) \{ (2q_0 z + 1)^{1/2} - 1 \}) \quad (2)$$

and the *K*-corrections are

$$K_1(z) = 2.5 \log_{10} \left[\frac{\int_0^\infty F(\lambda) S(\lambda) d\lambda}{\int_0^\infty F(\lambda/(1+z)) S(\lambda) d\lambda} \right] \quad (3)$$

$$K_2(z) = 2.5 \log_{10}(1 + z) \quad (4)$$

where $F(\lambda)$ is the radiation flux at wavelength λ and $S(\lambda)$ is the detector response. Following Oke and Sandage⁷ we have separated the effect of the redshift on the bandwidth, that is, the K_2 term, from the effect of simply translating to the redshifted wavelength, that is, the K_1 term. Our point is that for the U band, K_1 eventually starts decreasing with redshift, whereas K_2 increases monotonically. The essence of the present problem is the competition between these two terms.

For a deep plate, the detector response will depend on the film, its filter and the sky transmission. As the flux has only been measured for a few wavelengths, we have made the approximation

$$K_1(z) = 2.5 \log_{10} \left[\frac{F(\lambda_e)}{F(\lambda_e/(1+z))} \right] \quad (5)$$

with effective wavelengths λ_e of 0.33 μm for the U band and 0.44 μm for the B band. Using the curve in Fig. 1 together with

equations (1), (2), (4) and (5), we have calculated the apparent magnitude for the Sb/Sc galaxy at various redshifts. This was done for $q_0 = 0.2$ and for an absolute magnitude corresponding to the turn in the differential luminosity function. This occurs at $M_B^* = -21 \text{ mag } 1$ and $M_U^* = -21 \text{ mag } 2$ for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $U - B = -0 \text{ mag } 1$ (ref. 3). The results are shown in Fig. 2. A calculation with $q_0 = 0.02$ leads to little difference for redshifts below 1.0.

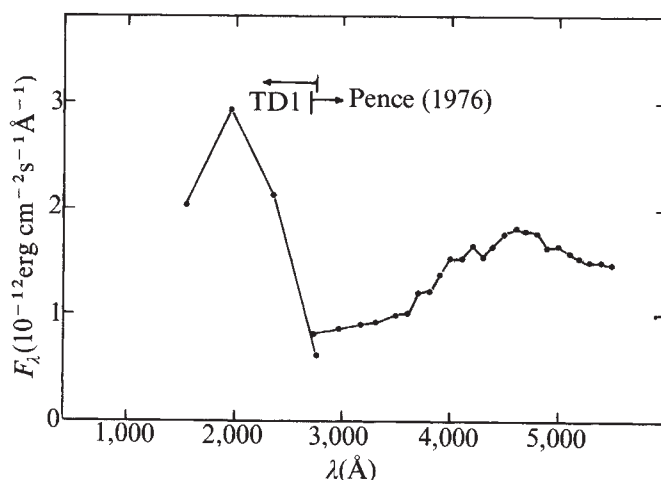
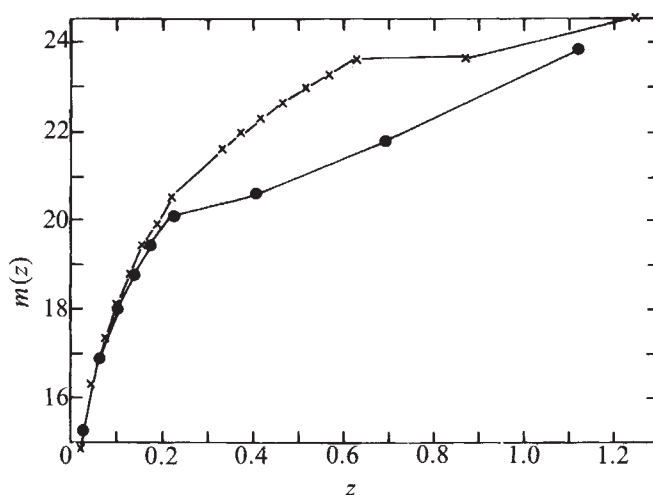


Fig. 1 Observed absolute spectral energy distribution for an Sb/Sc galaxy, normalised to *V* = 8 mag 49 (NGC 4258).

The distribution of visible galaxies with redshift on a U plate depends mainly on its limiting apparent magnitude. We have been unable to calculate this directly; instead we have arrived at an approximate value by comparing each factor involved with its appropriate counterpart when taking a deep B plate. The sensitivity of a B plate (nitrogen-soaked Kodak IIIa-J plus Schott GG395 filter) is close to that of a U plate (nitrogen-soaked Kodak

Fig. 2 Apparent U and B magnitudes at various redshifts. $\times$, $m_B(z)$; $\bullet$, $m_U(z)$.



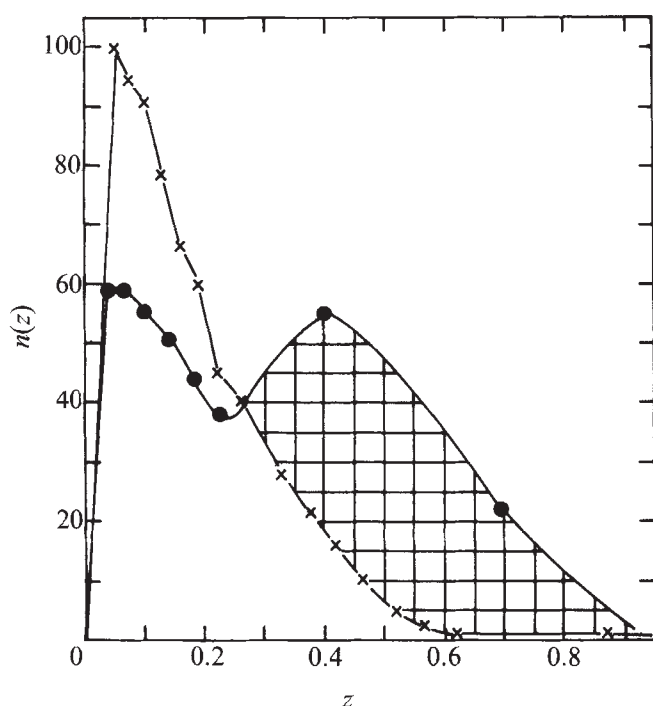


Fig. 3 Relative numbers of Sb and Sc galaxies visible on deep B and U plates at various redshifts. The hatched area represents the excess observed when blinking from the B plate to the U plate. ●, U plate; ×, B plate.

Ha-O plus Schott UG1 filter)^{8,9}. The major difference with deep U plates lies in the sky brightness. For the two wavelengths considered, Simkin¹⁰ quotes $U-B = -0.7$ mag. It has already been demonstrated that images as faint as $B = 23$ mag 0 have been recorded on IIIa-J photographs taken with the UK 48-in Schmidt. Hence we have considered here a limiting magnitude of $U = 22$ mag 3 as fairly realistic.

We can show that there is an excess of distant images on the U plate, as compared with those on a B plate. These could be readily identified by comparing the images on the two plates, for example via the COSMOS machine¹¹. In order to estimate how many galaxies would be on the U plate but not on the B plate, we have used Christensen's¹² absolute luminosity function. The loss of galaxies caused by atmospheric 'seeing' has been ignored. The effect of evolution has also been neglected, but it can be readily seen that the predicted time variation of the energy distributions¹³ will make the detection of distant galaxies even more favourable, mainly because distant, and thus younger, galaxies will be more luminous. The frequency of galaxies at various redshifts for plate limits $B = 23$ mag 0 and $U = 22$ mag 3 is plotted in Fig. 3. The excess shaded region on the graph corresponds to about 700 Sb and Sc galaxies for an area of 1 square degree, that is, about 15% of the total number of all types on 1 square degree of the B plate. The interesting feature is that most of these galaxies lie in the redshift range of 0.3–0.8.

As in the identification of QSOs, blinking between U and B plates can produce a host of other interlopers¹⁴, though the images sought here are close to the plate limit (Fig. 2); further comparisons with deep red plates might eliminate some of these. The fainter QSOs might be expected to have very large redshifts and neutral colours¹⁵ and hence appear on all three plates.

The suggestion depends on the approximate validity of the TD1 results. With respect to the discrepancy between the TD1 and OAO-2 results, we note that there is a similar discrepancy between the OAO-2 and the recent Skylab ultraviolet observations¹⁶. There is evidently an urgent need for further accurate extragalactic observations in the ultraviolet. Deep U plates have been taken recently with the UK 48-inch Schmidt telescope and

we intend to pursue the matter discussed in this note further using the COSMOS machine.

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The polarisation of nova Vulpeculae

MEASUREMENTS of the polarisation of nova Vulpeculae 1976 are reported. The linear polarisation seems to have an interstellar origin and so can be used to estimate the reddening and distance of the nova. Nova Vulpeculae 1976 was discovered at about the peak of its brightness on October 21 by the British amateur astronomer Mr Alcock.

Observations were made at the 90-inch (2.3-m) telescope of Steward Observatory on the evenings of October 25–27, 1976 using a dual-channel Pockels cell polarimeter with GaAs photomultipliers (RCA C31034A). Six passbands from 0.35 to 0.86 μm were defined with either interference filters or combinations of coloured glass filters and the spectral response of the photomultipliers. The details and results are given in Table 1 and Fig. 1. Quoted errors are 1σ from photon counting statistics.

The polarisation seems to have an interstellar, rather than intrinsic, origin. The wavelength dependence of linear polarisation is described well by Serkowski's empirical formula for interstellar polarisation¹, with the parameters $p_{\text{max}} = 3.35\%$ and $\lambda_{\text{max}} = 0.59 \mu\text{m}$ as shown in the figure. Furthermore λ_{max} and the position angle 12° (72° galactic) are typical for stars in this region^{1,2}. There is no evidence for (intrinsic) circular polarisation larger than 0.08% (3σ). Interstellar circular polarisation, however, is likely to be quite small; for example the circular polarisation of HD183143, a more strongly linearly polarised neighbouring

Fig. 1 Linear polarisation of nova Vulpeculae 1976. Curve is empirical formula for interstellar polarisation¹ with $p_{\text{max}} = 3.35\%$ and $\lambda_{\text{max}} = 0.59 \mu\text{m}$.

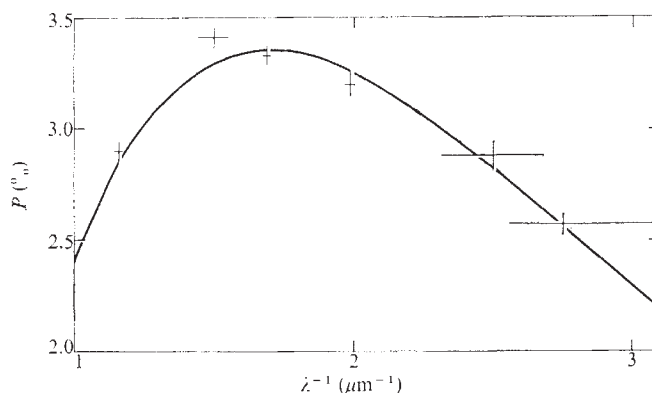


Table 1 Polarisation of nova Vulpeculae 1976

Date (Oct. 1976)	$\lambda^{-1} (\Delta\lambda^{-1} \mu^{-1})$	Linear	Polarisation (%) Position angle	Circular
25	2.75 (0.57)			0.03 $\pm$ 0.02
25	2.75 (0.57)	2.57 $\pm$ 0.04	12.2 $\pm$ 0.5	
27	2.50 (0.37)	2.88 $\pm$ 0.06	10.1 $\pm$ 0.6	
26	1.99 (0.03)	3.20 $\pm$ 0.06	11.2 $\pm$ 0.5	
26	1.69 (0.03)	3.33 $\pm$ 0.04	11.1 $\pm$ 0.3	
27	1.50 (0.11)	3.41 $\pm$ 0.04	12.5 $\pm$ 0.3	
26	1.16 (0.03)	2.90 $\pm$ 0.04	13.8 $\pm$ 0.4	
27	1.16 (0.03)			0.02 $\pm$ 0.02

star, is only about 0.01% (ref. 3).

If an interstellar origin is accepted, then the amount of polarisation may be used to estimate the reddening, the distance and thus the absolute magnitude (at maximum) of the nova. Hall² lists the colour excess E_{B-V} and the degree of polarisation p (at 0.45 μ m) for about two dozen stars within several degrees of the nova. The subset of these that are polarised about the same amount as the nova give p (%) $\approx (3.5 \pm 1.2 E_{B-V})$. For the nova $p(0.45 \mu\text{m}) = 3.1\%$, so that the estimated E_{B-V} is 0.9 ± 0.3 .

The same subset of stars range in distance modulus from 8.8 to 12.2, the average being about 11. The colour excess in this area of the sky is known⁴ to increase linearly with distance to 0.9 at 1 kpc, after which there is little further increase. Therefore a distance modulus of 11 ± 1 for the nova would be consistent with all of the data, though a larger value is possible.

The above estimates, together with a ratio of total to selective extinction of 3.3 and the reported $m_V = 6.5$ near maximum⁵, lead to $M_{V, \text{max}} = -7.5 \pm 1.5$, a reasonable value for a nova. This estimate should be compared to that which can be derived from the timescale of the decay of the nova light, when the latter becomes available.

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Radio 'jets'

FANAROFF and Riley¹ classified extragalactic radio sources according to whether high brightness radio hot spots are observed close to their outer extremities. A change in morphology is found when going from high (typically 10^{26} W Hz⁻¹ sr⁻¹) to relatively low (10^{24} W Hz⁻¹ sr⁻¹) luminosity at 178 MHz. The more luminous sources have pronounced outer hot spots and are all highly collimated double sources such as Cygnus A. On the other hand, their fainter cousins, which are less commonly found in radio surveys, contain no outer hot spots and comprise sources with relaxed double structure or having head-tail or complex morphologies. So far, little is known how these apparently different classes¹ of sources are related. In

particular, one might enquire whether the collimating process is less efficient in the nuclei of the fainter, more relaxed radio sources. Here we present new observations made with the Westerbork Synthesis Radio Telescope (WRST) which show that three of these relaxed type sources with intermediate 178 MHz luminosities have collimated structures emanating from their radio cores. Our high resolution observations show that these coherent structures are embedded in the otherwise smooth radio sources. These 'radio jets' may be similar in nature to the narrow structures recently observed (with less sensitivity) in the collimated double source 3C219 (ref. 2) and the complex radio galaxy 3C66B (ref. 3). We shall briefly discuss the consequences of these radio jets for models of radio sources.

Table 1 Observing parameters

Source	Observing frequency (MHz)	Observing dates	Half-power beamwidth (RA $\times$ dec) (arc s)	Effective noise at field centre (10^{-29} W Hz ⁻¹ m ⁻²)
	4995	14 Jan. 76	6 $\times$ 12	0.8
		16 Jan. 76		
B0844+31	1415	1 July 76	22 $\times$ 42	1.0
	610	Dec. 72–May 73	52 $\times$ 98	4.6
	4995	4 May 73	6 $\times$ 14	0.8
3C310		18 May 73		
	1415	14 May 71	22 $\times$ 50	1.0
		Dec. 72–Feb. 73		
3C129	4995		6 $\times$ 9	0.6

New observations were made at 5 GHz of the relaxed double galaxies B0844+31 and 3C310 and of the head-tail galaxy 3C129. B0844+31 was originally classed as a QSO but the high resolution observations of Grueff and Vigotti⁴ showed that this was a misidentification. In fact the source is associated with a 15.5 magnitude giant elliptical galaxy⁵. The morphology of 3C310 was discussed by Miley and van der Laan⁶ and 3C129 is one of the most studied head-tail galaxies^{7,8}.

The Westerbork Telescope is described elsewhere^{9,10} and relevant parameters pertaining to the present observations are shown in Table 1. Contour maps of the 5 GHz total intensities are shown in Figs 1–3 side by side with lower resolution Westerbork maps made at 1.4 GHz and

Table 2 5-GHz flux densities (10^{-29} W Hz⁻¹ m⁻²) of jets and other features in the sources

Source	Jet	Extended	Core	Total	Redshift
B0844+31 North	75	44	58	250*	0.0675 ⁽⁹⁾
South	28	45			
3C310 North	40	1045	95	1280†	0.0540 ⁽²¹⁾
South	100				
3C129 North	260	1626	14	2160†	0.0210 ⁽²¹⁾
South	290				

*Estimate from fringe amplitudes at shortest baseline.

†Single dish observations (ref. 21).

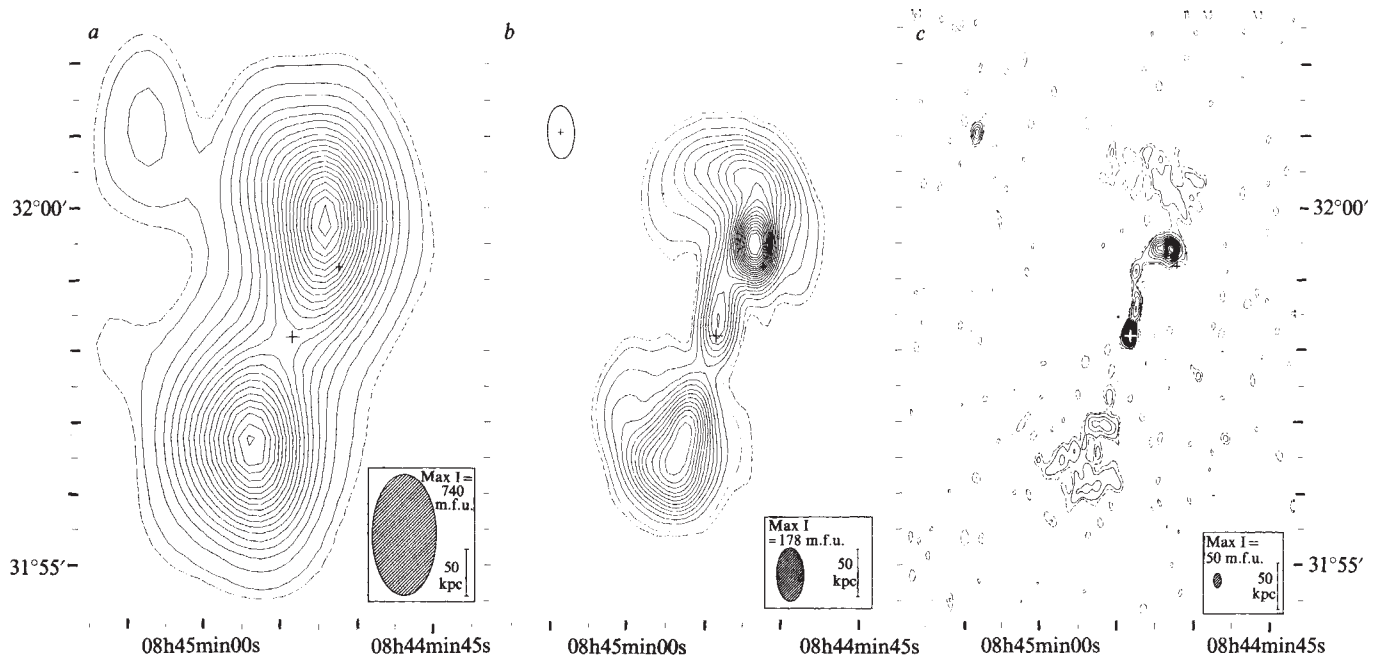


Fig. 1 Total intensity distribution of B0844+31 at 0.6, 1.4 and 5 GHz. Continuous contours are plotted at 5% intervals from 5% to the indicated value of MAX. I. Long dashed contours represent the 3% level and short dashed contours the -5% level. The shaded ellipse represents the half-power level of the synthesised beam and the crosses the position of the galaxy and the QSO. In *b*, an unresolved (presumed background) source is subtracted. Also indicated are the relevant linear scales.

(in the case of B0844+31) at 0.6 GHz. All maps are corrected for primary beam attenuation. The linear scales were obtained using the redshifts listed in Table 2 and a Hubble constant of $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The cross at the central radio component indicates the identified galaxy. For B0844+31, the QSO (at the edge of the northern hot spot in Fig. 1c) is also indicated as well as a subtracted (presumed background) point source in the north-east part of Fig. 1b.

Note the relaxed appearances of B0844+31 and 3C310 apparent in the lower resolution maps (Figs 1a, b and 2a).

By contrast, the 5 GHz observations show a considerable amount of small scale structure. Both sources contain compact radio cores with relatively flat radio spectra coincident with the visible galaxies. Most remarkable are the highly collimated elongated structures emanating from these cores. This is most pronounced in the case of B0844+31 but present to a lesser extent in the southern part of 3C310. From the latter source, however, another characteristic seems to show up more clearly: comparison of Fig. 2a and b suggests that the coherent, elongated structure is embedded in a larger region of low brightness.

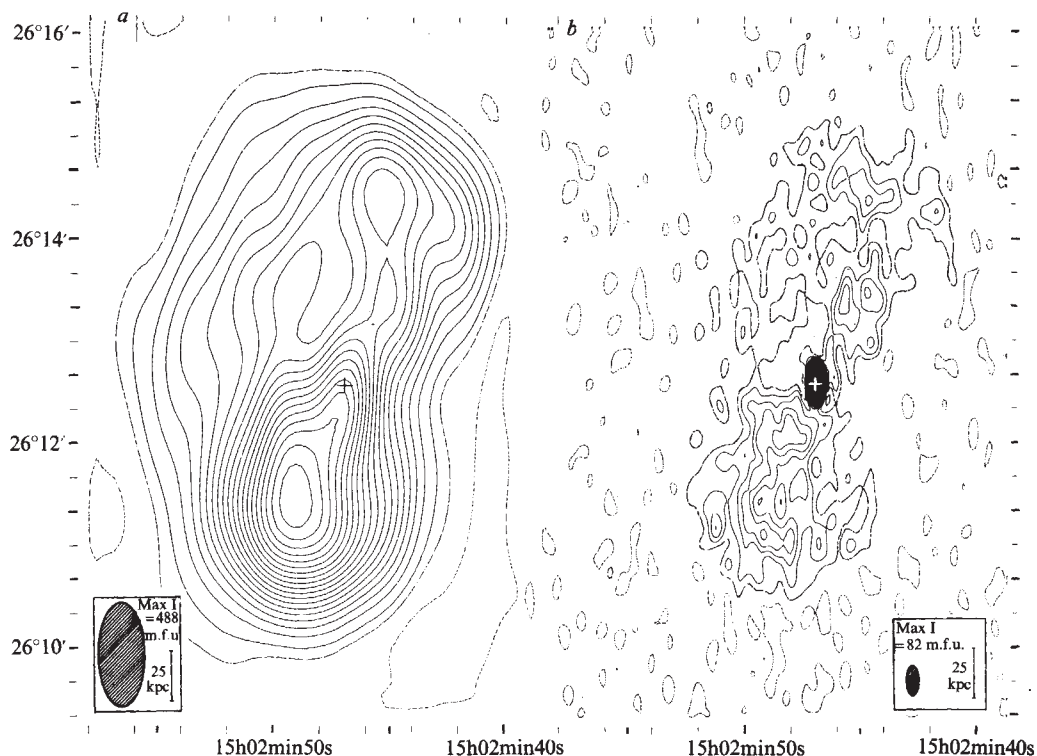


Fig. 2 Total intensity distribution of 3C310 at 1.4 GHz and 5 GHz. Low intensity contours are 2, 5 and 10% in *a* and from 2 to 10% in intervals of 2% in *b*. Further contours are as in Fig. 1. Negative contours are -2% (short dashed). The +2% contours are long dashed.

The radio jet in the northern part of B0844+31 terminates in a region of relatively high brightness situated about half way to the edge of the source. A weaker hot spot is apparent to the south. Beyond the hot spots the emission is relatively uncollimated.

Although the jets are very narrow they are not straight. One can distinguish 'wiggles' on several different scales. For both sources the shape of the wiggles is suggestive of an S-type symmetry¹¹ close to the radio cores. However, from Fig. 1b, the outer parts of the northern and southern components of B0844+31 have relatively sharp gradients to the west and tail off gradually to the east so that the large scale structure of this source may exhibit a C-type symmetry¹¹.

It is interesting to compare the morphologies of these two relaxed double sources with that of the head-tail source 3C129. Figure 3 shows an unpublished 5 GHz map of the head made with the WSRT. Again there is a compact radio core near the centre of the visible galaxy. According to the radio trail hypothesis¹² the head-tail morphology is formed when a 'normal' double radio source is distorted by the motion of its parent galaxy through a dense intergalactic medium. Without this distortion one might expect the head of 3C129 to resemble the central part of B0844+31. The pair of highly elongated arms stretching back to the north-west are then analogous to the radio jets in Figs 1 and 2. The set of parallel wiggles in the two arms of 3C129 would correspond to the S symmetry in the jets of the relaxed double radio sources. (In the case of 3C129 these wiggles can be traced a distance of several hundred kiloparsecs along the tail.)

Other possible examples of radio galaxies with jets are 3C83.1 (NGC 1265) (ref. 13), 3G130 (ref. 14), 3C402 (ref. 6), 3C449 (ref. 14), 3C465 (ref. 6), Virgo A (ref. 15), Centaurus A (ref. 16), and NGC 315 (ref. 17). When most of these relatively relaxed sources are observed with sufficient sensitivity and linear resolution better than a few tens of kiloparsecs, jet-like features are seen protruding from their radio cores into more extended, low brightness regions. This indicates that although these sources are relatively relaxed, they still have a very efficient collimating process in their nuclei.

Table 2 compares the observed flux densities for the jets with those of other features in the sources. An examination of the data available for the sources presented here together with those of possible candidates indicate that the radio jets typically have 5-GHz luminosities of 10^{22} W Hz⁻¹ sr⁻¹ and sizes of a few tens of kiloparsecs. The contribution of the jet to the total flux is small, so that for beam widths much greater than the jet widths (a few kiloparsecs), their structure is 'smeared out' over the more extended low brightness regions. The detection of the jets therefore requires a combination of sufficient sensitivity and linear resolving power. The collimated Cygnus A type sources are on average about a hundred times further away and with currently available instruments it is impossible to say whether they also have comparable jets protruding from their cores. However, the 2-kpc component within 3C236 (ref. 18) and the jet component in 3C219 (ref. 2) may represent similar but more powerful phenomena.

How are these radio jets to be interpreted within the framework of current theoretical efforts? In recent years a large body of evidence has built up in favour of models of highly collimated double sources in which energy is supplied quasi-continuously from an active nucleus (at the radio core) to the outer components. The energy is transported by a succession of ejected plasmoids or by a relativistic beam. The existence of the jets suggests that such quasi-continuous energy transport also takes place in the weaker relaxed sources. One can envisage the jet as a tunnel along which the energy is channelled. The hot spots at the outer edges of the collimated doubles have their

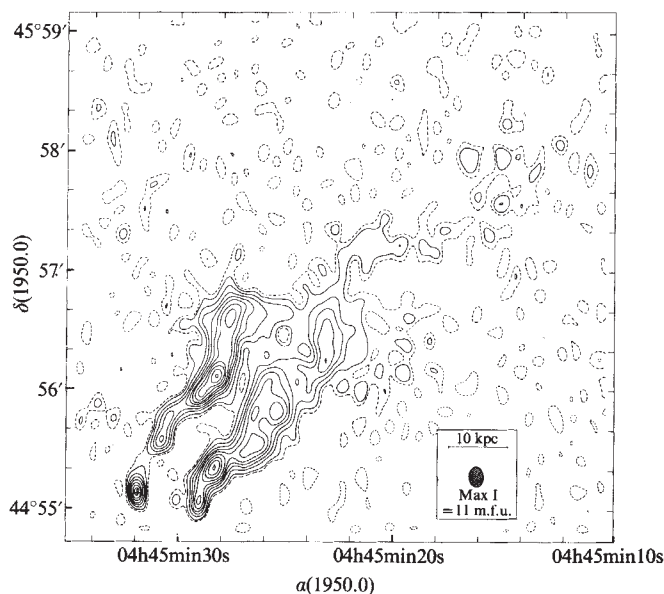


Fig. 3 Total intensity distribution of 3C129 at 5 GHz. Continuous contours are plotted at 10% intervals from 10% to the indicated value of Max. I. A 5% contour (long dashed) and -10% contour (short dashed) are added.

counterparts in the regions of enhanced radio brightness where the tunnels terminate. In these regions the energy density builds up and results in enhanced synchrotron emission. In the less luminous sources the hot spots are found relatively close to the core. Possibly, since less energy is involved, in this case the energy transport is more influenced by conditions within the parent galaxy.

Of particular interest are the S-shaped wiggles in the jets. They appear to occur on a scale about half that of the jets themselves, corresponding roughly to the size of the associated visible galaxies. This correspondence could be indicative of a hydromagnetic instability due to the interaction of the galaxy with the ambient medium. Alternatively the wiggles may be caused by a change in the orientation of the nuclear powerhouse during the source lifetime^{19,20}. Such a wobble in the collimation axis could account for the symmetries apparently seen in B0844+31, 3C310 and 3C129 as well as in several other sources¹⁹. The C-type symmetry in the large scale structure of B0844+31 (Fig. 1b) is most probably due to translational motion of the source through an ambient medium.

Analysis of the spectral and polarisation data for these sources may give more insight into the exact role that the jets play in the energy transport process. In the coming year the sensitivity of the WSRT, at 5 GHz, will be improved by a factor of ten. Further effort will then be made to find more sources with radio jets.

We thank our colleagues at Westerbork, Dwingeloo and Leiden who made these observations possible and acknowledge useful discussions with Professor van der Laan. The Westerbork Synthesis Telescope is operated by the Netherlands Foundation for Radio Astronomy with financial support of the Netherlands Organisation for the Advancement of Pure Research (ZWO).

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Frequency of nearby supernovae and climatic and biological catastrophes

MCCREA^{1,2} has proposed that the encounter of the Solar System with a dense cloud of interstellar material during its passage through a spiral arm of the Galaxy may produce such climatic catastrophes on Earth as the ice epochs. His thesis, an extension of several earlier investigations^{3,4,5}, is based on the expected effect on the solar constant of an increased accretion rate. Unfortunately, the cloud density necessary to produce the required variation is 10^5 to 10^7 cm⁻³, and although clouds with such extreme densities are thought to exist⁶, Begelman and Rees⁷ point out that they are so compact and unusual as to reduce the likelihood of the Sun ever encountering one. (Dennison and Mansfield's suggestion⁸ that there is no evidence for the existence of a nearby super-dense cloud which could have caused the most recent ice epoch has been answered by McCrea⁹.) Begelman and Rees⁷ in fact showed that a much more modest cloud density ($\approx 10^2$ to 10^3 cm⁻³) would prevent the solar wind from reaching the Earth, with resulting modification of the near-Earth environment; however, the climate consequences of such a modification are not well understood. We present here an alternative hypothesis which still retains McCrea's association with the passage of the Solar System through a spiral arm, but which relates the initiation of an ice epoch, plus biological catastrophes, to an encounter of the Solar System with a nearby supernova outburst rather than a super-dense interstellar cloud.

The spiral structure of our Galaxy indicates the regions where bright, short-lived stars illuminate the interstellar gas and dust. The two principal spiral arms are believed to reflect a self-sustaining density-wave pattern through which the disk of stars and gas move. Observations indicate dust lanes along the inner (concave) edge of each arm, and this is interpreted as a shock-wave effect whereby clouds become temporarily compressed as they cross the lane. A cloud of interstellar matter in its orbit round the Galaxy is liable to enter a spiral arm by way of its compression lane about once in $\approx 10^8$ yr. In general the cloud material will re-expand on emerging from the lane after $\approx 10^6$ yr (the cloud not necessarily retaining its original identity). But sometimes a cloud is compressed to a condition for star formation, and the result is a new galactic cluster which proceeds to traverse the associated spiral arm itself, taking $\approx 10^7$ yr to do so. If this cluster contains a star of mass some $10M_\odot$ or greater it would be expected to evolve rapidly over less than $\approx 10^7$ yr, terminating in a supernova outburst of Type II before emerging from the spiral arm in which it is formed. In this view supernovae of Type II would occur only in spiral arms (or near galactic nuclei), in accord with observation. Indeed, a 'supernova zone' might be defined beyond the compression lane of a spiral arm; such a zone is depicted diagrammatically in Fig. 1, in the assumption of a $20M_\odot$ stellar lifetime of $\approx 5 \times 10^6$ yr and a transverse velocity (at about the solar distance from the galactic centre) of 200 km s^{-1} giving a zone width of $\approx 1 \text{ kpc}$.

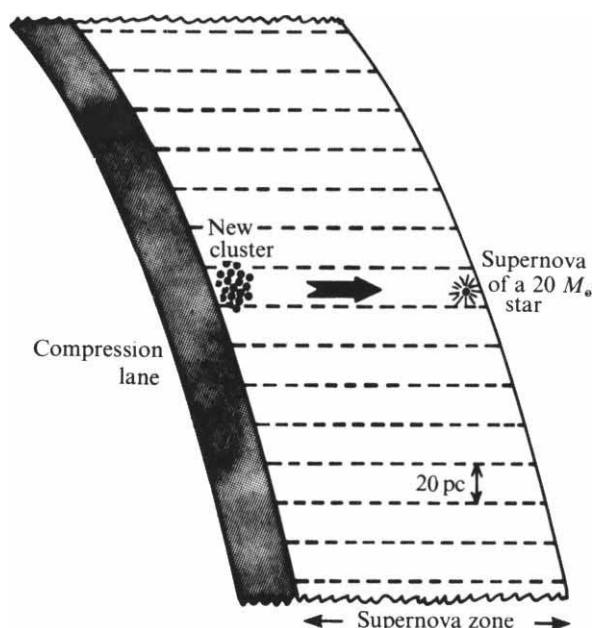
The occurrence rate of supernovae within the Galaxy is still only poorly determined. At least five historically observed supernovae

occurring on the near-side of the Galaxy during the past millennium^{10,11} would seem to place a lower limit on their frequency throughout the Galaxy of about one per 100 yr. It must, however, be stressed that the short interval between some actual sightings of supernovae can give a grossly misleading impression of the frequency of events because of the extreme distances involved. For example, the AD 1006 supernova occurred at a distance of about 3,000 light years from the Earth¹², while that of AD 1054 occurred at a distance of over 6,000 light years (ref. 12), so that although first sighted on Earth within half a century of each other the outbursts were actually separated in time by over 3,000 yr. Clark and Caswell¹³ have concluded from a statistical analysis of the radio remnants of supernovae that the average interval between outbursts leaving long-lived observable remnants (that is, those lying within spiral arms and evolving only slowly) is ≈ 100 yr. Shklovsky¹⁴ prefers an interval of a mere 30 yr for Type I (population 2) events, but accepts a longer interval of ≈ 100 yr for those of Type II (population 1). Results from other investigators range between these extremes. In the absence of a more definite estimate, we will take an average interval between Type II supernovae of 100 yr.

If there is one Type II supernova per 100 yr, and if, as seems reasonable, this is localised to one of the 'supernova zones' proposed above, then this implies 5×10^4 such supernovae in one (of two) spiral arms in a period of $\approx 10^7$ yr (the time for the Sun to traverse a spiral arm). Since a spiral arm is $\approx 2 \times 10^4$ pc long, this suggests that 50 supernovae will occur in a 20-pc strip of a spiral arm's 'supernova zone' each time the Sun crosses it. Thus at least one supernova would be expected to occur within 10 pc of the Sun each time it crosses a spiral arm (that is, about once every 10^8 yr). Note that this simple calculation takes no account of the thickness of a spiral arm perpendicular to the plane; however, since the bulk of supernova remnants are concentrated to within a few tens of pc of the plane¹⁵, this simplification is not expected to increase the above estimate for the interval between nearby supernovae by more than a factor of two.

Estimates of the radiation flash¹⁵ emitted (mainly in γ and X rays) in a supernova explosion vary from $\approx 10^{47}$ to 10^{50} erg. (This represents a very small fraction of the total energy of the outburst of $\approx 10^{52}$ erg.) Assuming the lower value, the peak integrated energy flux expected above the Earth's atmosphere from a supernova at ≈ 10 -pc distance is $\approx 10^6 \text{ erg cm}^{-2}$ (this compares with the solar flux of $\approx 20 \text{ erg cm}^{-2} \text{ s}^{-1}$ in the wavelength range of interest). Absorption in the upper atmosphere means that this flash gives no significant surface radiation dose initially. Nevertheless, Ruderman¹⁵ has predicted catastrophic effects on the Earth's protective ozone layer. The ionising radiation from the supernova

Fig. 1 Schematic representation of a supernova zone (not to scale).



flash produces excited free nitrogen atoms in the atmosphere which quickly oxidise. The oxides of nitrogen catalytically destroy ozone, and Ruderman claims that by this mechanism the ozone layer density may be temporary depleted. Such destruction of the ozone layer would expose the Earth's surface, and more particularly life on it during the past 600 Myr, to lethal solar ultraviolet radiation.

The expanding shell of ejecta from a supernova at 10-pc distance would reach the Earth after about 2,000 yr, with the passage of the shell taking several hundred years. During this latter period the density of primary cosmic rays would be expected to be enhanced by perhaps a factor of a hundred¹⁴. About one third of the background radioactivity level at the Earth's surface is contributed by cosmic radiation (the remainder being due to terrestrial factors). Thus a hundredfold increase in cosmic ray intensity would produce a drastic thirtyfold increase in mean level of radioactivity. In addition, the enhanced cosmic-ray flux would be expected to produce the same destruction of the ozone layer as produced by the initial radiation flash. The possible biological consequences of such dramatic increases in background radioactivity and ultraviolet radiation fluxes expected for protracted periods some time after a nearby supernova outburst may have been catastrophic for certain species. Ruderman¹⁵ has suggested that in man the huge increase in ultraviolet radiation would give rise to a vast increase in the incidence of skin cancer; in animals with calcified internal skeletons the increased vitamin D production might have been toxic.

The supernova-initiated destruction of the ozone layer would be expected to have climatic as well as biological consequences. Whether ozone removal would heat or cool the Earth is unclear; the main argument for cooling is that ozone contributes (in the 2- μ m region) significantly to the greenhouse effect, so that its removal could lower the Earth's temperature and thus possibly initiate an ice age.

The question arises as to whether one would be able to observe the nearby remnant of the supernova which might have initiated the last major ice age $\approx 10^5$ yr ago. Unfortunately, after such a period the remnant of this supernova would already have reached its extinction phase¹³, and would almost certainly be impossible to identify with certainty.

The possible astronomical influences on the Earth's climatic and biological history must remain a matter of speculation. Nevertheless, we suggest that the evidence that the Solar System encounters a nearby supernova (that is closer than 10 pc) on average once per orbit of the Galaxy must be included in any future consideration of these topics.

Note added in proof: In a recent note, Whitten *et al.*¹⁷ have independently considered the effects of a nearby supernova on atmospheric ozone. From a consideration of the galactic distribution of supernova remnants given by Clark and Caswell¹³, they concluded that the probability of a supernova occurring within 10 pc of the Earth during the past 10^8 yr is only 1–5%. Our somewhat greater estimate of a 50% probability during any 10^8 -yr period is a consequence of our confining (at least Type II) supernovae to the galactic spiral arms, in accord with the observational evidence^{12,14}, and assuming that within the spiral arms the rate of occurrence of supernovae is independent of distance from the galactic centre.

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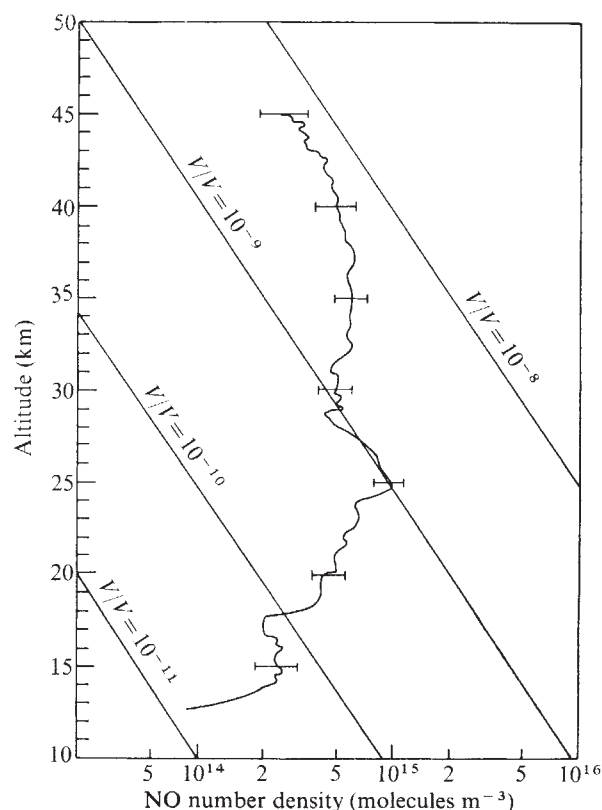
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Balloon-borne chemiluminescent measurement of NO to 45 km

MEASUREMENT of nitric oxide (NO) above an altitude of 40 km is of interest because in this region of the stratosphere >70% of the total odd nitrogen ($\text{NO} + \text{NO}_2 + \text{HNO}_3 + \text{N}_2\text{O}_5$) is thought to exist as NO, and at 45 km the NO is predicted to be approximately 90% of the total¹. This measurement thus provides an important test for odd nitrogen concentrations as predicted by theoretical models for ozone chemistry. Some models show that of the total ozone destruction necessary to explain the observed ozone concentration, approximately 50–70% is due to the odd nitrogen catalytic cycles².

We have made direct measurements of the nitric oxide (NO) concentration between 12 and 45 km with a balloon-borne chemiluminescent detector. This is the first balloon-borne chemiluminescent measurement to an altitude of

Fig. 1 Vertical profile of the nitric oxide concentrations for the June 27, 1976, balloon flight from Sioux Falls, South Dakota (1 atmosphere $43^\circ 26' \text{N}$). The instrument was launched at 1108 GMT and the data were taken on ascent. The local Sun times for the data at 12 km and 45 km were approximately 0530 and 0730, respectively. The marked increase in NO at 18 km correlated with the altitude of the stratospheric wind reversal. The altitude of the tropopause was 10.5 km.



45 km. The data above 40 km are particularly useful to test theoretical models of ozone chemistry because above 40 km most of the odd nitrogen is thought to be in the form of NO. The data from a balloon flown on June 27, 1976, from Sioux Falls, South Dakota (Fig. 1), show a nearly constant mixing ratio of 6.5 p.p.b.v. from 40 to 45 km, with a possible peak value at 43 km. One other high-altitude stratospheric NO measurement has been made by a rocket-borne chemiluminescent detector but the data were taken quickly (in 25 s) and without on-board flow measurements, zeroing, or calibration³. Several chemiluminescent devices, interferometers, spectrometers, and lasers have been flown on balloons to a maximum altitude of 35 km (refs 4–7). These measurements indicate a large range in NO concentrations which suggests that transport plays a strong role for odd nitrogen and thus ozone chemistry in the lower stratosphere.

Our instrument was similar in principle to other chemiluminescent NO detectors⁸, and a brief description of the device follows. Ozone for the photon producing reaction $\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + h\nu$ was carried as ozone absorbed on silica gel at dry ice temperatures. The O_3 source was servoed to produce a constant concentration of O_3 in the reaction vessel at all ambient pressures. The sample gas was drawn through a 1.2-m pyrex tube and heated to 30 °C before entering the gold-plated reaction vessel. A positive displacement blower was specially developed to provide a constant 31-s^{-1} flow rate at all altitudes encountered during the flight. The blower exhausted the sample gas-ozone mixture through a 3-m flexible tube located on the side of the instrument opposite the sample inlet tube. The balloon ascent provided a 15-km-h^{-1} vertical flow rate so that the exhausted ozone did not pollute the sample. The instrument was suspended 100 m below the balloon and flight control package. Zero NO signal levels were taken every 40 s by destroying the NO in the sample gas with ozone upstream of the reaction vessel. The instrument was calibrated every 2.5 min by pumping NO (in N_2) calibration gas into the sample inlet tube.

The data for the ascent are shown in Fig. 1. The mixing ratio from 40 to 45 km is essentially constant at 6.5 p.p.b.v. with a possible peak at 43 km. The results show considerable structure in the NO concentration in the lower stratosphere. There is a marked increase in NO mixing ratio at 18 km which corresponds to the altitude of the wind reversal for that day. Where data can be compared, our results show general agreement with other measurements^{1,9}.

The funding for the 15×10^6 cubic foot balloon and the balloon launching crew (we thank Raven Industries) was provided by the United States Office of Naval Research.

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Sea surface temperature related to rain in Ceará, north-eastern Brazil

RAINFALL in Ceará is correlated with sea surface temperature in the south Atlantic Ocean. Knowledge of sea surface temperature makes possible a useful rainfall forecast before the rainy season begins. The mechanism may be that sea temperature affects the height of the trade wind inversion, and thus the height of the moist layer. The data also suggest in association between below normal sea temperatures in the south Atlantic and the El Niño in the Pacific.

Ceará is the node of Brazil's drought-prone north-east. Here severe drought, and its antithesis, flooding, both occur about once per decade, arriving without warning. The human misery that follows in their wake might be reduced if they could be forecast. We found that sea surface temperature (SST) in the south Atlantic Ocean and rainfall in Ceará are correlated in time, and if SST is known, a useful preliminary rainfall forecast can be made.

Brazilian climatologists have long believed rain in the north-east to be related to fluctuations in SST, but have been unable to show such a relationship owing to lack of sufficient SST reports. We investigated this relation by using a data file containing all available surface marine weather reports from ships of many nations. This file, Tape Data Family -11, was assembled by NOAA's National Climatic Center. Working in conjunction with the Fleet Numerical Weather Central and the North Pacific Experiment (NORPAX) the National Marine Fisheries Service has used the tape data file to compute mean sea surface temperatures by 5° latitude-longitude quadrants for each month in the period October 1947 to December 1967. We used these computations to map the correlation coefficient between SST and a rainfall index in Ceará. Later, after we had identified a key 5° quadrant where correlation was particularly high, monthly SSTs were recomputed for the period 1907–72, and used in a regression analysis to develop a forecasting equation. Our results may be somewhat biased by the selection of this particular quadrant, and further research may show that improved forecasts can be made using data from other areas.

The density of SST observations is far from uniform over the South Atlantic. Reports are most abundant along shipping lanes between the Cape of Good Hope and Cape Verde, and along the South American coast. Data are extremely sparse over the central South Atlantic, and are not sufficient to map correlation coefficients over the entire area. Data are not available in all quadrants in all years, and different years are missing from different quadrants. Nevertheless, when considered as a whole, the data form a picture that is coherent and of predictive value.

The rains in Ceará are highly seasonal, about 40% coming in January–February–March, and about half in April–May–June. Almost no rainfall occurs in the months August to November. Fortaleza and Quixeramobim are two weather stations in Ceará about 120 km apart that have long series of rainfall observations extending back to 1849 and 1896, respectively. We correlated monthly mean SST data with rainfall at both stations, but found that a combined index of simultaneous data from both stations gave higher correlation coefficients. The combined index smooths local variations of rainfall to emphasise large scale influences affecting both stations. Since the mean annual rainfall at Fortaleza, on the coast, is about twice that of Quixeramobim, in the interior, we gave each station equal weight in the index by using twice Quixeramobim's rainfall plus Fortaleza's.

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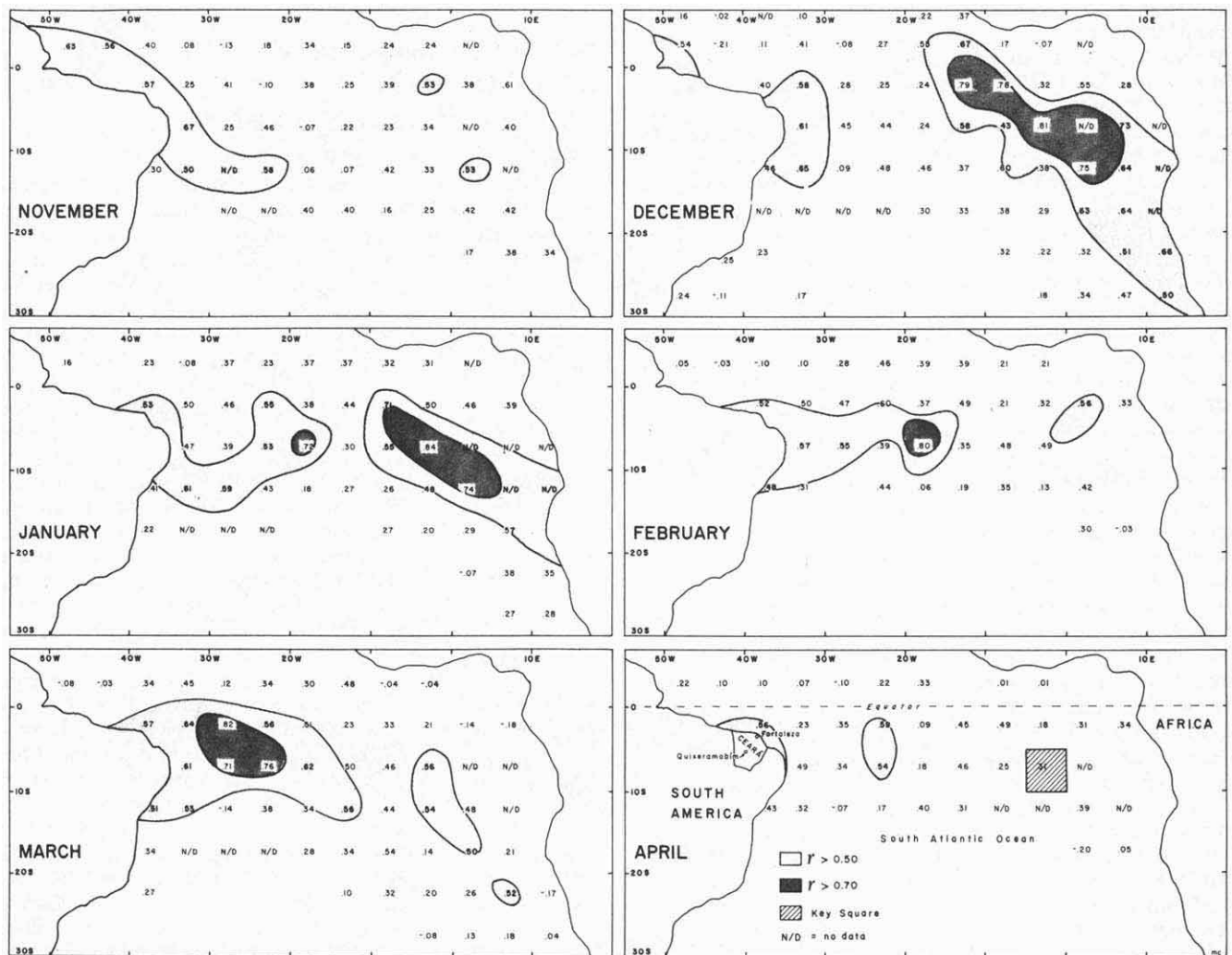


Fig. 1 Correlation between SST and January-February-March rainfall indices in Ceará. An area of high coefficient of correlation appears off the African coast in November, becomes strong in December, moves westward with the South Equatorial Current during January, February and March, and disappears in April.

We computed correlation coefficients (r) for each of the 5° quadrants in the South Atlantic for which data are available, between the monthly values of SST and the rainfall index for the combined months of January-February-March, as well as for the annual rainfall indices. SSTs for November and December were correlated with rainfall indices for the coming year, while SSTs for January through April were correlated with the current year.

Maps of correlation coefficients (Fig. 1) show an apparent westward movement of high correlation during November to April. This may be related to the westward drift of water in the South Equatorial Current. In December the quadrant where correlation is highest is centred at $7^\circ 30'S$, $2^\circ 30'W$ (Marsden Square 300, Quadrant 3). For this area r was 0.81 for January-February-March rainfall, and 0.73 for annual rainfall. Ceará tends to be dry when water here is cold, and wet when it is warm. Moreover, since December precedes the rainy season in Ceará, December SST can be used to forecast rainfall with useful lead time. Thus we selected Quadrant 300-3 as the key area for regression analysis with Ceará's rainfall.

The 1947-67 data used for our correlation maps are means of all reports within a given 5° quadrant and thus might be biased by clustered reports. This possible bias can be reduced by making individual means for each 1° area, then averaging the 1° means to obtain a 5° mean. This

latter procedure was used to obtain data points for our long term regression analysis (Fig. 2).

We noticed a long term warming trend of $0.51^\circ C$ between the years of 1907 and 1972, in our SST means at Quadrant 300-3. The trend could be caused by real warming, or by differences in measurement techniques that have occurred over the period. If it is assumed that the latter is true, and if the effects of the trend are removed from the regression, r for the period increases from 0.61 to 0.66, Kendall's rank-order coefficient, τ increases from 0.20 to 0.41. This increase in order through detrending suggests that the apparent trend is instrumental and not a real warming.

Depending on the sets of data, r values range from 0.61 in the 1907-72 data uncorrected for trend, to 0.81 in the 1947-67 data used in making the correlation maps. Values of τ range from 0.21 to 0.50. All are for correlation between December SST and the Ceará rainfall index for the following January-February-March period. If we assume r to be 0.71, then r^2 , the coefficient of determination, is 0.50, indicating that half of Ceará's January-February-March rainfall can be predicted by mean SST in Quadrant 300-3 in December. If we assume $\tau=0.40$, we can say that the odds are 70/30 that a change in SST will be followed by a change in rainfall index of the same sign. Our rainfall forecast ranges from 500 mm with an SST of $22^\circ C$ to 2300 mm

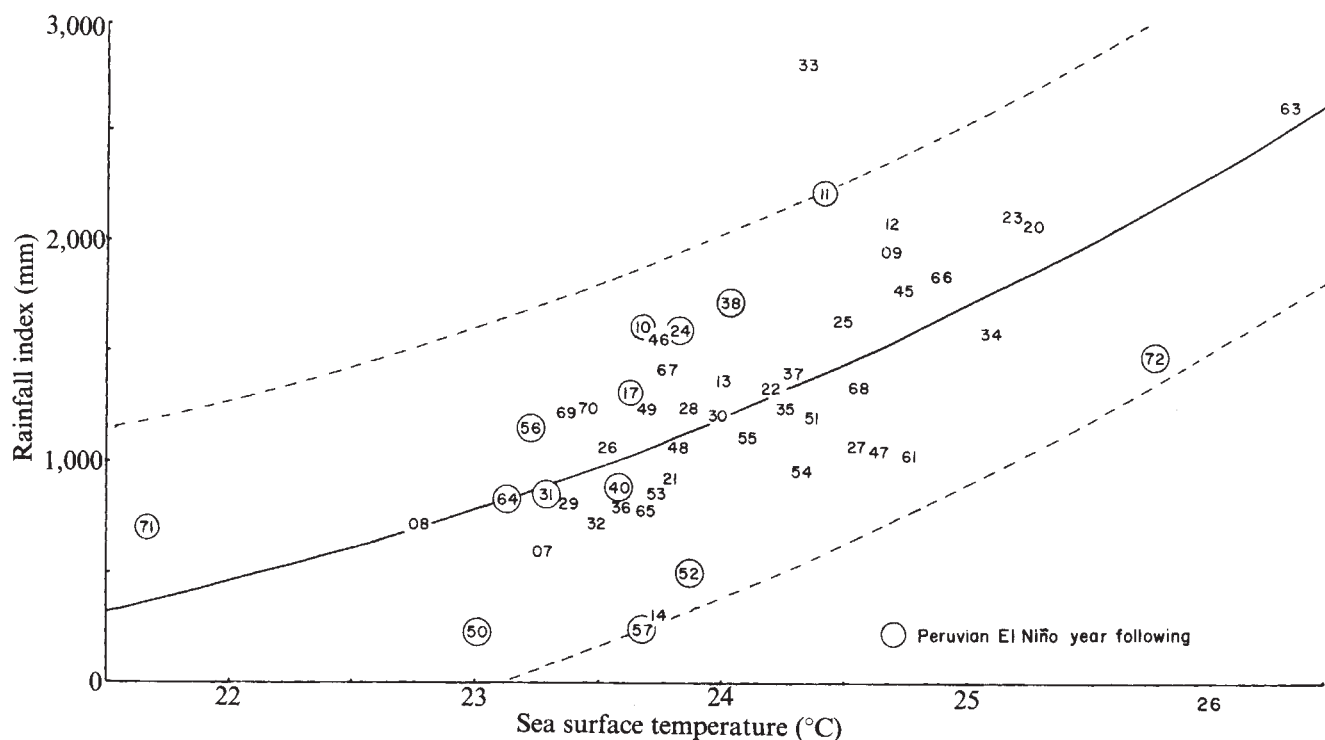


Fig. 2 January-February-March rainfall indices in Ceará plotted against December SST in Marsden Square 300, Quadrant 3. Years indicated are for December SST values. Data has been detrended by adding 0.0078°C times the number of years before 1972. Second degree regression lines and 95% confidence limits are shown. El Niño years (taken from Caviedes (1973) and Quinn⁶) are circled.

	1907-72 Detrended	1907-72 Not detrended	1947-67 From Fig. 1
<i>n</i>	53	53	16
<i>r</i>	0.66	0.61	0.81
α	0.001	0.001	0.001
τ	0.41	0.20	0.50
α	0.001	0.04	0.007

with an SST of 26°C ; a considerable improvement over the climatic normal value of 1267 mm. If we define drought as the lack of rainfall and flooding as an excess of it, from Fig. 2 we can state that drought is unlikely when SSTs at Quadrant 300-3 are warmer than 24.5°C , and that flooding is unlikely when SSTs are colder than 23.5°C . If we are given some particular SST value, say 24.0°C , our best estimate for the rainfall index is 1200 mm, and we can say with 95% confidence that it will fall between 350 and 2050 mm. These are much wider limits than we should like but suggest that eastern Atlantic SST in December, albeit important, is only one of a number of factors influencing Ceará's rainfall.

A suggested mechanism for the correlation is as follows: cold water stabilises the overlying air and decreases wind flow and convection over the Atlantic. The trade wind inversion represents the boundary between subsiding air from aloft and convective air from below. When convection is weak and winds are light, the trade wind inversion is lowered, and the thickness of the layer carrying moisture into north-eastern Brazil is reduced. Similarly, warm seas strengthen wind flow, and mixing and increased convection raise the trade inversion to deepen the moist layer. Further, the Intertropical Convergence Zone (ITC) and its attendant raininess would tend to lie in the region of warmest water, and would retreat from the hemisphere with cold water.

Namias¹ found intense cyclonic activity near Newfoundland to be highly correlated with rain at Quixeramobim. He hypothesises the following chain of events. The North Atlantic Subtropical High is strengthened when the trough in the Newfoundland land area deepens. The trough is held in place by blocking toward the east. The strengthened sub-

tropical High increases the north-east trade winds which in turn require the south-east trades to respond by strengthening, thus increasing the flow of moisture into Ceará. An alternative explanation for Namias' correlation is its reverse. Warm South Atlantic sea temperatures produce heavy equatorial rains. These release large quantities of latent heat, strengthening the Hadley circulation in both hemispheres, and inducing a blocking High in the North Atlantic. The increased flow from the Equator, accompanied by the blocking, results in increased cyclonic activity in the Newfoundland area, with a concurrent deepening of the trough. To support this latter explanation, Markham² found that a strong South Atlantic High and strong south-east trades seem to decrease, rather than increase, rain in Ceará. The rainiest periods do not occur when the south-east trades are strong, but when the High is weak or has retreated toward the south, allowing flow with a northerly component to penetrate Ceará. There is little doubt that the Newfoundland trough and rain in Ceará related, but which is the cause and which the effect is unclear. In any event, precipitation over northeastern Brazil is important to the general circulation because it represents a significant energy input to the atmosphere, and with large variations from year to year. The magnitude of this variation is shown by the annual rainfall index which varied from a low of 884 mm in 1915 to a high of 5505 mm in 1964.

From the work of Namias³ and Bjerknes¹, a body of literature on large scale ocean-atmospheric interactions is growing. Recently Caviedes⁴ has related drought in north-eastern Brazil with the El Niño—the appearance of anomalously warm water off the Peruvian coast. Temperatures in Quadrant 300-3 also seem to be related to El Niño. The years preceding El Niño are circled in Fig. 2.

The hypothesis that cold December SSTs precede El Niño has a one-tailed significance level of about 0.0125 when the non-parametric Wilcoxon two-sample test is applied⁷. The coldest December temperature in Quadrant 300-3, 21.67 °C in 1971, was followed by an El Niño of exceptional severity in 1972. The El Niño of 1972 may have persisted into 1973 and thus may account for the anomalous point for 1972 circled in Fig. 2. If this did occur, the correlation is greatly improved. Quinn⁶ has related El Niño to the difference in surface pressures between Easter Island and Darwin, Australia. This difference is an index of the Southern Oscillation and suggests that the occurrence of warm water off Peru simultaneously with cold water off Africa is related to the Southern Oscillation. The mechanisms for this phenomenon are poorly understood.

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Upper Jurassic pyroclastic rocks in Skye, west Scotland

DURING a systematic sampling of shale lithologies in the Jurassic beds of Skye, an examination was made of sections in the Staffin Shales¹⁻³ in Staffin Bay. Two localities were visited, one immediately south of the landslip at point 6 (ref. 4) [NG470712] and the other at point 7 (ref. 2) [NG468715]. Six shale samples were collected at the former section, representing the *Kosmoceras jason* and *Erymnoceras coronatum* zones (Middle Callovian), the *Peltoceras athleta* and *Quenstedoceras lamberti* zones (Upper Callovian), and the *Quenstedoceras mariae* and *Cardioceras cordatum* zones (Lower Oxfordian). Four shale samples were collected from the section at point 7, representing the *Ringsteadia pseudocordata* zone (Upper Oxfordian) and the *Pictonia baylei*, *Rasenia cymodoce*, and *Aulacostephanus mutabilis* zones (Lower Kimmeridgian).

The shale samples were thus representative of the bulk of the sequence, but further samples were collected from two of three distinctive pale grey mudstone bands present in the point 6 section. The lowest of these mudstones (here termed the *athleta* band) lies about 0.75 m above the base of the shales of the *Athleta* zone. The upper two bands, about 1.7 m apart, could not be zonally dated from field observations alone, but foraminiferans from the lower band have been identified by Dr A. W. Medd as belonging to the lower part of the *Cordatum* zone (*Bukowskii* or *Costicardia* subzone) of the Lower Oxfordian. As the upper part of the *Cordatum* zone (*Cordatum* subzone) is at least 4.8 m thick¹, the upper band must also belong to the *Cordatum* zone.

The upper two mudstone bands are therefore referred to as the lower and upper *cordatum* bands, respectively.

Petrographical examination of the shale samples has revealed the presence of minor amounts of pyroclastic material, as discussed below. More striking evidence of volcanic ash-fall is, however, provided by the mudstone bands. These are internally structureless apart from mottling due to burrows. They shrink and break into small angular fragments on drying at room temperature and on subsequent immersion in water they rapidly swell and disintegrate to a flocculent mass of clay and a residue of fine particulate material. The residual particles consist largely of irregular grains composed of isotropic material, chlorite or calcite. Thin sections reveal a poorly defined normal grading of these grains, some of which display elliptical structures similar to amygdalites in basic volcanic glass. Accessory grains include euhedral or fractured-euhedral prismatic apatite crystals and fresh euhedral or angular-anhedral flakes of biotite. By contrast the associated shales contain a typical detrital assemblage of abundant quartz, with minor feldspar, muscovite, chlorite and biotite.

X-ray diffraction analysis shows the clay fraction to consist of pure montmorillonite in the lower *cordatum* band and montmorillonite and kaolinite in the *athleta* band. The clay fractions of the associated shales in both cases consist principally of mixed-layer montmorillonite-illite and kaolinite.

In their physical properties and mineral composition these mudstones are comparable with some of the well-documented American bentonites⁴, and there can be little doubt that the mudstones represent tuffs which have undergone diagenetic alteration to bentonitic clays. The absence of quartz and zircon in the residues suggests that the tuffs were not acidic and the absence of attenuated shard structure in the glass particles also implies a basic or intermediate composition. The presence of biotite indicates that the source lavas were at least in part of andesitic or trachytic type. Feldspars are absent in both of the mudstone bands but it is probable that scattered patches of coarsely crystalline calcite represent the replacement of primary feldspar crystals.

Undoubted volcanic material (altered glass particles and euhedral apatite crystals) also occurs in minor amounts in otherwise 'normal' detrital shale samples from the *Coronatum*, *Athleta*, *Lamberti*, *Mariae* and *Pseudocordatum* zones (the *Plicatilis*, *Transversarium* and *Cautisnigrae* zones were not sampled). Altered glass particles also seem to be present in the *Baylei* and *Cymodoce* zones of the Lower Kimmeridgian, although they are too finely grained to display diagnostic amygdaloidal textures. No volcanic constituents have been recognised in samples of the *Mutabilis* zone of the Kimmeridgian or of the *Jason* zone of the Middle Callovian.

Microscopic examination of Bajocian and Bathonian shales collected in the same area has not revealed any recognisable volcanic components, although the abundance of montmorillonite in some beds is suggestive of a volcanic contribution⁶. Thus although volcanism may have contributed finely divided ash to the Middle Jurassic sediments, it would appear that a phase of stronger volcanic influence started in the Middle Callovian and lasted at least until the Lower Kimmeridgian. Phases of maximum activity during the Upper Callovian and Oxfordian are indicated by the altered tuff bands.

Jurassic volcanicity in the continental shelf area around Britain is now well established with the discovery of lavas and tuffs in Middle Jurassic beds of the Piper-Forties area of the North Sea⁷. These volcanics are dated as Bathonian and Bajocian. Another volcanic centre or centres to the south-west of Britain may have been the source of volcanic components of the Bathonian Fuller's Earth of the Bristol district⁸, and perhaps also of the montmorillonite bands in

the Bathonian of the East Midlands⁶. Upper Jurassic (?Kimmeridgian) tuffs have been recorded from the Brent area of the North Sea⁷, and a possible tuff reported in the Kimmeridgian beds of Andøy, Norway⁸.

If its inferred Jurassic age is correct, the Piper-Forties volcanic centre cannot have been the source of the Upper Jurassic pyroclastic deposits of Skye, although it is possible that the North Sea eruptions are represented in Skye by the high proportion of montmorillonite in Middle Jurassic beds (ranging from the Dun Caan Shales, Bajocian (Aalenian), *Ludwigia murichsonae* zone to the Ostracod Shales, ?Lower Bathonian).

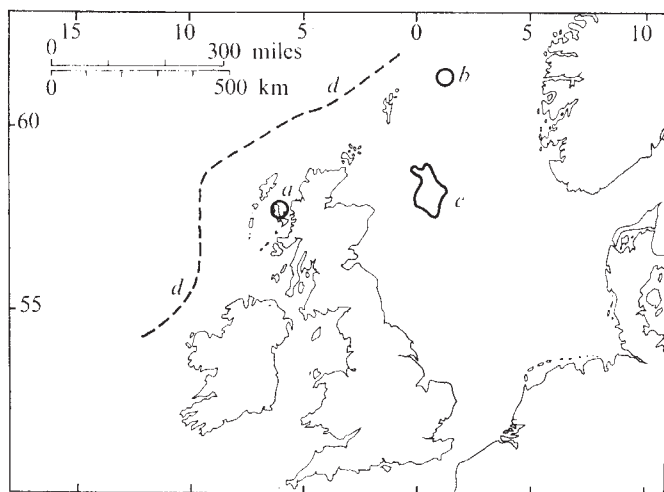


Fig. 1 Location of Upper Jurassic tuffs in Skye (a) and in the Brent area of the North Sea (b). The Piper-Forties volcanic centre (c) is essentially of Middle Jurassic age and Upper Jurassic volcanism may have occurred in a zone of potential rifting (d) to the west and north of Britain.

A possible alternative source area for the Upper Jurassic tuffs is the zone now represented by the series of NE-SW troughs⁹ which follow the present-day shelf margin to the west and north of Scotland. The closest of these, the Rockall Trough, may not have developed until Cretaceous times but a period of initial rifting and volcanism may have begun as early as the Middle Jurassic¹¹. With the limited information at present available it seems most likely that the volcanic source for the Skye and perhaps Brent tuffs lay within this zone of potential late Jurassic rifting (Fig. 1).

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An unusual electromagnetic surface force

IN a recent experiment¹ a disk of high dielectric constant material (barium titanate) was suspended as a torsional pendulum in an intense axial magnetic field. It was found that if a radial electric field was applied to the disk in time quadrature with the magnetic field a substantial torque was produced which, to within experimental accuracy (<5%), was in agreement with a force density $\dot{\mathbf{P}} \times \mu_0 \mathbf{H}$ in the disk. The surprising feature of the torque was that it had a time average, contradicting the 'Abraham force' which predicts a force density of $\partial(\mathbf{P} \times \mu_0 \mathbf{H})/\partial t$ with zero time average. The experiment seemed to suggest that the quantity $\dot{\mathbf{P}} \times \mu_0 \mathbf{H}$ does not produce a force since its time average would be equal but of opposite sign to the time average of $\mathbf{P} \times \mu_0 \dot{\mathbf{H}}$.

In order to obtain a high electric field strength in the dielectric a thin metal coating was evaporated on to the outside edge of the disk and the edge of the central hole. The electrical resistance of these coatings was sufficiently high so that induced currents due to the time-varying magnetic field had a negligible effect on the total electric and magnetic fields. As a check, cuts were made in the coatings, thereby preventing any circulating current flow, with no observable change in the behaviour of the system.

On further investigation it seems that the metal coatings have a most unexpected effect, as may be seen from the following argument. Suppose that air gaps are created between the coatings and the dielectric surfaces, the gaps being small enough to cause no significant perturbation of the electromagnetic fields. Three mathematical closed surfaces can be described, one just outside and surrounding the dielectric and the other two just outside and surrounding the metal coatings. The moment of the electromagnetic surface stress for each surface can be obtained from the Maxwell tensor by evaluating the integral

$$\int \mathbf{r} \times [\epsilon_0 \mathbf{E} (\mathbf{E} \cdot \mathbf{n}) + \mu_0 \mathbf{H} (\mathbf{H} \cdot \mathbf{n}) - \frac{1}{2} (\epsilon_0 E^2 + \mu_0 H^2) \mathbf{n}] dS$$

For the surface enclosing the dielectric it may be shown that this has zero time average but for the surfaces enclosing the metal coatings the moment reduces to $\int \mathbf{r} (\epsilon_0 \mathbf{E}_t) E_0 dS$ where the latter is taken over the parts of the mathematical surfaces in the air gaps. When this is evaluated the measured value of torque results. The interpretation is that an electrical charge of density $\epsilon_0 E$ is induced on the surface of the coating adjacent to the dielectric and a drag action is exerted on this surface charge by the azimuthal electric field component E_0 .

In light of this, the experiment verifies that the total mechanical force on the dielectric has no time average, as is consistent with the Abraham force. That the term $\mathbf{P} \times \mu_0 \dot{\mathbf{H}}$ produces a force can be understood by considering the Kelvin force $(\mathbf{P} \cdot \nabla) \mathbf{E}$ which may be written $\frac{1}{2} (\epsilon - \epsilon_0) \nabla E^2 + \mathbf{P} \times \mu_0 \dot{\mathbf{H}}$. In the above experiment $\mathbf{E}$ is independent of θ and so ∇E^2 does not contribute to the torque. A further conclusion to be drawn is that the experiment is consistent with a force density in the dielectric given by $\dot{\mathbf{P}} \times \mu_0 \mathbf{H} + (\mathbf{P} \cdot \nabla) \mathbf{E}$ provided $\mathbf{E}$ is the macroscopic internal field and not a 'local' field as proposed in different forms by a number of authors.

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Insect growth regulator and sterile males for suppression of horn flies

A RECENT trend in the control of insects has been the development of pest management systems whereby combinations of control methods, applied to one or more stages of the life cycle, increase efficiency and optimise effectiveness of individual methods. The potential of an integrated programme involving the release of sterile insects in combination with other control methods was discussed by Knipling¹. He pointed out that two properly chosen and implemented systems might be regarded as synergistic in effect. Kunz and Eschle² have proposed an integrated programme against the horn fly, *Haematobia irritans* (L.), and they have suppressed the reproduction of a semi-isolated population 98% by releasing sterilised horn flies³. In the spring of 1973, we began a pilot test on the island of Molokai, Hawaii. The objective was to develop a control system whereby a natural population of horn flies could be suppressed by chemical means and then eradicated through the release of sterile flies.

Harris *et al.*⁴ have demonstrated that the development of horn flies is inhibited in the faeces of cattle fed methoprene (isopropyl (*E,E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), an insect growth regulator (IGR). Because methoprene would affect only the reproduction of the native population, it could be used simultaneously with releases of sterile insects. In a preliminary experiment on the Kalaupapa peninsula of Molokai, continuous treatment of the drinking water of cattle with methoprene for 6 weeks reduced the estimated number of horn flies from 360 to seven per animal⁵.

We report here the elimination of a wild population of horn flies through the simultaneous application of the IGR and the release of sterile males. The experiment was begun March 1975 on Puu-O-Hoku Ranch (POH), a 3,000-acre

ranch with improved pasture and about 900 cattle (cows and calves), primarily Charolais and some Hereford.

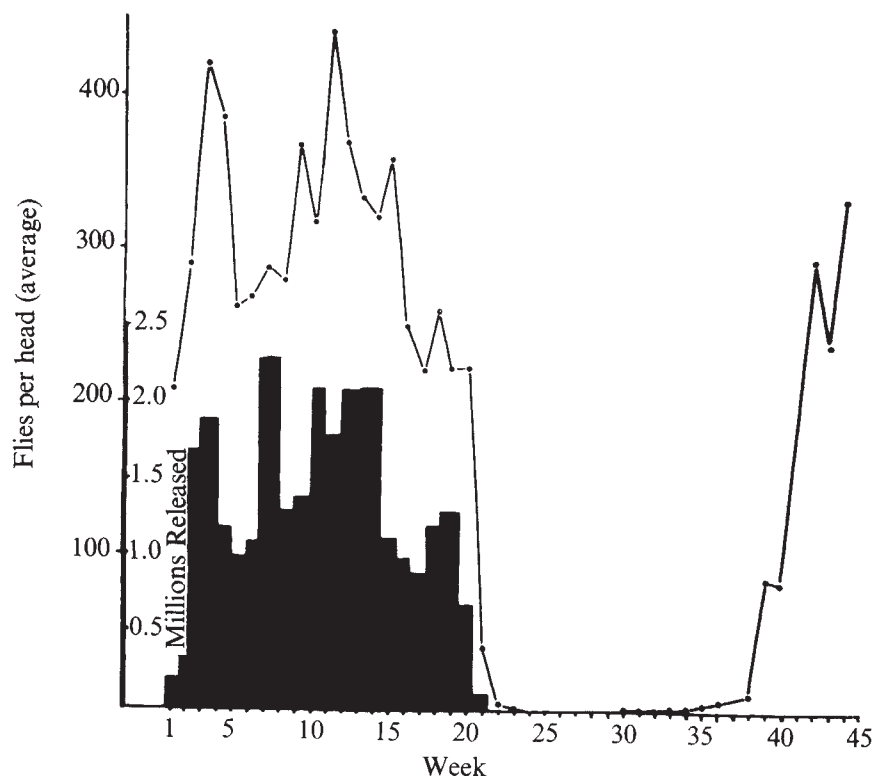
POH is at the extreme eastern end of Molokai. The north-easterly trade wind, prevalent throughout the year in Hawaii, has a mean flow of 20 knots at POH and through the Pailolo channel that separates Molokai from Maui; it blows an average 90% of the time during April to September⁶. Theoretically, the test site was thus afforded some degree of protection from reinfestation by horn flies moving from cattle on other parts of Molokai and also on the island of Maui 9 miles to the south-east. When we released about 1.5×10^6 marked horn flies on the west end of Maui, none were recovered from cattle on Molokai. The only movement of marked flies observed when releases were made on Molokai was from east to west and from north-east to south-west.

The experiment was conducted on the north-eastern section of POH only (about 750 cattle) so that all other cattle on Molokai were downwind from the test area. South-west POH was not included because the pastures there contained streams which were impractical to treat with the IGR.

Methoprene was added to the drinking water of the test cattle as a tablet formulation. An automatic dispensing system⁷ was attached to the outlet of the float-controlled valves of each water trough. Thus, the water was maintained with a concentration of 0.05–0.1 p.p.m. of methoprene, a level in the drinking water adequate to inhibit development of the horn fly in the manure of treated cattle⁷. Levels of methoprene in water samples from selected troughs were determined by gas chromatography. Effectiveness of treatments was confirmed by a bioassay in which samples of field-collected manure were seeded with horn fly eggs.

The sterile horn flies were provided from two colonies, one at Kerrville and another in Hawaii on the island of Oahu⁸. Chilled adult flies were sterilised with 2,500 rad (¹³⁷Cs source in Kerrville and ⁶⁰Co in Hawaii),

Fig. 1 Numbers of sterile horn flies released per week (bar graph) and estimated numbers of adult horn flies per head of cattle (line graph) on Puu-O-Hoku Ranch (March 17, 1975 to January 18, 1976).



marked with a fluorescent pigment, and placed in perforated paper bags. The bags were sealed in insulated containers and shipped by air to Molokai (in transit about 30 h from Kerrville and 16 h from Oahu). During shipment the flies were maintained in a chilled state by frozen eutectic material; O₂ was provided to sustain the lower metabolism, and an absorbent was used to avoid accumulation of CO₂.

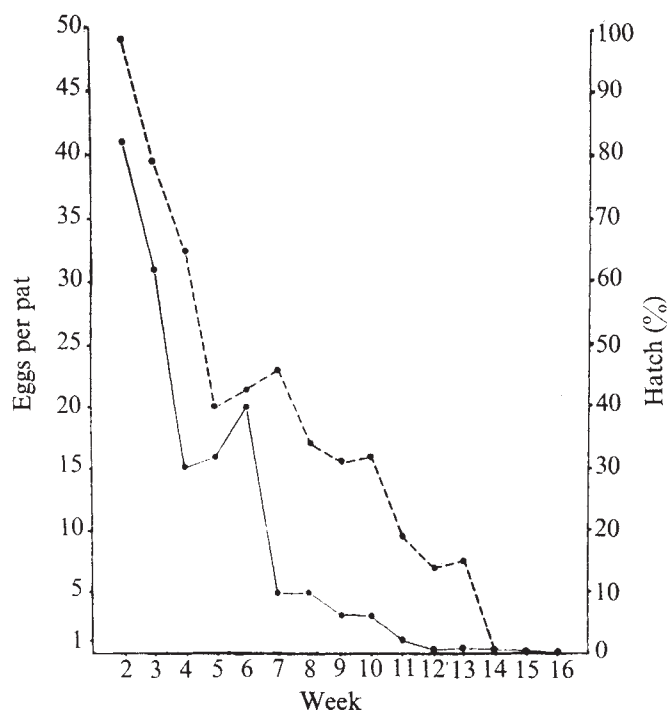
Sterile flies were released daily in the vicinity of cattle. Although released flies were marked, we could not consistently obtain samples of adult flies from test cattle to determine the ratio of released to native flies. Therefore, the frequency and rate of release in each pasture were determined by the number of flies available, the need for sterile flies as indicated by the current sterility of eggs sampled from the pasture, and the estimated number of adult horn flies on the cattle. From the volume (ml) of dead flies that remained 24 h after release, we estimated that more than 80% of the sterilised flies escaped from the release boxes.

The combined effect of the IGR and release of sterile flies were assessed from estimates of numbers of adult horn flies per animal and from numbers and fertility of horn fly eggs collected from manure pats in test pastures³.

Methoprene treatment began on March 13–14, and release of sterile flies 1 week later (week 1). For the first 14 weeks of the experiment an average of 1.6×10^6 sterile flies per week were released. The population of adult flies (Fig. 1) averaged 300 per animal for weeks 1 to 14. But a steady decline in numbers and fertility of eggs, from an average 41 eggs per pat and 98% hatch during week 2 to 0.4 eggs per pat and complete sterility during week 14, indicated a decline in the population of native horn flies (Fig. 2). Therefore, we reduced the number of sterile flies released by about half (an average of slightly more than 800,000 per week). Beginning on week 16, no horn fly eggs were found on any of the pats in spite of intensified examinations; this indicated that the native population had been eliminated.

Release of sterile flies in the test pastures was discontinued after week 21. To prevent reinfestation, however,

Fig. 2 Effect of combined use of methoprene and the sterile-male technique on production and fertility of eggs from horn flies on Puu-O-Hoku Ranch (March 24 to July 6, 1975). —, Eggs per pat; ---, per cent hatched.



we released the bulk of sterile flies on cattle in south-west POH and made periodic, low-level releases in a buffer zone (pastures on the south-west border of the test area). Three weeks after the last release and for the next 6 weeks no adult horn flies were found on the cattle nor eggs in the manure. Meanwhile we continued to find a few, mostly sterile, eggs in the buffer zone, an indication that releases there were effective. Nevertheless, reinfestation occurred during week 30 when cattle from south-west POH were moved into the test area without our knowledge. By week 39, native populations had become re-established.

All treatments were discontinued on POH during week 39. At that time we were devoting more attention to treatments in the buffer zone, and on south-west POH and other parts of Molokai. Thus we were unable to maintain the methoprene treatments as carefully as during the initial weeks of the experiment. The concentration of methoprene in water samples was found to be inadequate (<0.05 p.p.m.) in five of six troughs sampled in the test area during week 39. The population of adult horn flies had increased to an average 333 per animal by week 44 (Fig. 1).

The experiment demonstrated that methoprene in combination with releases of sterile males can be used to eliminate a native population of horn flies. Furthermore, the results suggested a strategy that could be used to implement a suppression-eradication programme involving a larger area such as the entire island of Molokai and, ultimately, the entire state of Hawaii. The control system could be used in a stepwise sweep from one end of the island to the other; the sweep would be planned so as to take advantage of natural isolating factors such as wind and geographical characteristics. A major advantage of such a strategy is that the size of the control area in each step could be controlled conveniently to fit the available personnel and mass rearing capabilities.

Implementation of the system on a large scale would require further refinements in rearing methods, the registration of methoprene for use in drinking water of cattle, and an understanding of the economic benefits.

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Y chromosome controls mating behaviour on *Anopheles* mosquitoes

SEVERAL species of *Culex* and *Anopheles* mosquitoes differ in their ability to breed in a relatively small space under laboratory conditions. When paired matings or mass rearings are carried out in small laboratory cages, males of some species are able to copulate while those of others are not. These characteristics have been named respectively stenogamy and eurygamy and reflect the field requirement for a small or a large space to perform the pre-copulative

Table 1 Results of paired matings between *Anopheles atroparvus* × *Anopheles labranchiae* hybrids with different sex chromosome combinations

Cross no.	Parents ♂	Parents ♀	Offspring code	Sex chromosomes in the male parent	Stenogamy in the male parent	Total	Paired matings No. fertile	No. sterile
1	<i>A. a.</i>	<i>A. a.</i>	<i>A. a.</i>	X _a Y _a	yes	10	10	0
2*	<i>A. l.</i>	<i>A. l.</i>	<i>A. l.</i>	X _l Y _l	no	10	10	0
3	<i>A. a.</i>	<i>A. l.</i>	I	X _a Y _a	yes	19	19	0
4‡	I	I	II	X _l Y _a	yes	9	1	8
5§	I	<i>A. a.</i>	III	X _l Y _a	yes	—	—	—
6	<i>A. a.</i>	I	IV	X _a Y _a	yes	11	11	0
7¶	IV	IV	V	X _a Y _a ; X _l Y _a	yes	10	6	4
8	IV	<i>A. a.</i>	VI	X _a Y _a ; X _l Y _a	yes	13	6	7
9	<i>A. a.</i>	IV	VII	X _a Y _a	yes	9	9	0
10*	<i>A. l.</i>	I	VIII	X _l Y _l	no	7	7	0
11§	VIII	<i>A. a.</i>	IX	X _a Y _l ; X _l Y _l	no	15	6	9
12§	VIII	VIII	X	X _a Y _l ; X _l Y _l	no	7	4	3
13	<i>A. a.</i>	VIII	XI	X _a Y _a	yes	10	10	0
14§	<i>A. l.</i>	<i>A. a.</i>	XII	X _l Y _l	no	6	6	0
15§	XII	XII	XIII	X _a Y _l	no	2	0	2
16	<i>A. a.</i>	XII	XIV	X _a Y _a	yes	12	12	0
17	XIV	XIV	XV	X _l Y _a ; X _a Y _a	yes	8	4	4
18§	<i>A. l.</i>	XII	XVI	X _l Y _l	no	5	5	0
19*	XVI	XVI	XVII	X _l Y _l ; X _a Y _l	no	11	4	7

A. a. = *Anopheles atroparvus*; *A. l.* = *Anopheles labranchiae*. The subscripts *a* and *l* under X and Y indicate their origin from *A. a.* and *A. l.* respectively. The *A. a.* strain has been reared in the laboratory for 20 yr. The *A. l.* mosquitoes were captured in the North of Sardinia as 3rd or 4th instar larvae. All crosses were performed in 20-cm cubic cages kept at room temperature of 25–28 °C, with humidity of 80–90%, in an undarkened room. Crosses were made by paired mating, using males at least 5 d old and females to which one or two blood meals had been given. Mass rearing was performed in the same type of cages with the number of mosquitoes given in parentheses: cross 5 (58 ♂ × 21 ♀); cross 7 (80 ♂ × 40 ♀); cross 11 (7 ♂ × 5 ♀); cross 12 (50 ♂ × 25 ♀); cross 14 (257 ♂ × 126 ♀); cross 15 (23 ♂ × 18 ♀); cross 18 (5 ♂ × 3 ♀).

*Artificial insemination.

‡ Only one female offspring was reared from this cross.

§ Mass rearing resulted sterile.

¶ Mass rearing resulted fertile.

flight': in stenogamous species the male performs an almost stationary flight with short, quick movements followed by mating with the female which remains motionless on a wall; conversely, the males of eurygamous species form small swarms of several individuals into or near which the females fly and copulation takes place in flight. These behavioural characteristics are inherited and, although the dominance of stenogamy over eurygamy has been suggested²⁻⁵, previous data are inconclusive mainly because of the lack of informative F₂ and backcross experiments⁶. In fact, in *Anopheles*, though all types of F₁ hybrid females are fertile when backcrossed to the parental species, the F₁ hybrid males are usually sterile⁶.

To investigate the genetic control of the eurygamy/stenogamy polymorphism we did a series of crosses between the sibling species of the genus *Anopheles*, *A. labranchiae*, which is eurygamous and *A. atroparvus* which is stenogamous, using artificial insemination⁶ whenever the male was unable to copulate because of its stenogamous behaviour. For comparison we also performed a number of mass rearings. We found that the polymorphism is controlled by the Y chromosome.

The results of our crosses are shown in Table 1, in which the basic character taken into consideration is stenogamy in the male parent. These experiments first confirmed the sterility of all males which are hybrids for the sex chromosomes irrespective of their autosomal constitution; this is obvious from crosses 4, 5 and 15. Whenever the sex chromosomes belong to the same species, the male is fertile, as seen from crosses 6, 9, 10, 13, 16 and 18. Furthermore, when segregation for the X chromosomes of the two species is expected in the male progeny and these males are crossed, a corresponding 1:1 ratio of sterile and fertile paired matings is found, as in crosses 7, 8, 11, 12, 17 and 19. We made histological and meiotic studies of the sterile males and found that the sterility is due to arrest of spermatogenesis before first meiotic metaphase.

Whenever the male carries the Y chromosome of *A. atroparvus* the mating behaviour is stenogamous and copulation takes place spontaneously in cages, as shown

by crosses 1, 3, 4, 5, 6, 7, 8, 9, 13, 16 and 17. Conversely, all males with the Y chromosome of *A. labranchiae* are eurygamous (see crosses 2, 10, 11, 12, 14, 15, 18 and 19) and artificial insemination was necessary for their reproduction.

Reproductive success of the male is therefore controlled on the one hand by the Y chromosome which determines mating behaviour and on the other by testicular fertility which is determined by the constitution of the XY pair, as exemplified in Table 2.

Table 2 Sex chromosome constitution controls testicular fertility and pre-copulative flight behaviour in *A. atroparvus* (a) × *A. labranchiae* (l) hybrids

	Testicular sterility	Testicular fertility
Eurygamy	X _a Y _l	X _l Y _l
Stenogamy	X _l Y _a	X _a Y _a

In conclusion, the behavioural polymorphism stenogamy-eurygamy is obviously controlled by one or more genes located on the Y chromosome. To our knowledge this is the first example of direct chromosomal control of a behavioural characteristic involved in reproduction.

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Cadmium tolerance in populations of *Agrostis tenuis* and *Festuca ovina*

It is well known that plant populations growing on soils contaminated with heavy metals are genetically tolerant to those metals. This adaptation is not found in populations growing on normal soils (ref. 1 and review in ref. 2). Most of the literature is concerned with the resistance of plants to Pb, Zn and Cu²⁺, but Cd salts too are often present in the soils of areas polluted by those metals. I have therefore investigated the occurrence of Cd tolerance in some Belgian and German populations of *Agrostis tenuis* Sibth. and *Festuca ovina* L. *sensu lato*.

Population samples of at least 25 individuals of *A. tenuis* and *F. ovina* were collected from sites contaminated with heavy metals and from normal sites, and transplanted for

non-toxic solutions. In each population 25 individual tillers were rooted in distilled water and the longest root of each was measured. The rooted tillers were then transferred to heavy metal solutions at different concentrations; a test sample was maintained in distilled water. After 5 d the longest root was measured again. The ratio

$$100 \times \frac{\text{length of the longest root in toxic solution}}{\text{length of the longest root in non-toxic solution}}$$

is known as 'the tolerance index' of the population for a given metal at a specific concentration. The same procedure was used to study the effects of soil nutrients (Ca, Mg, K and Na added as sulphate or nitrate) on the tolerance level of plant populations.

In the samples from normal areas, we found small amounts of total Cd (1.0 mg kg⁻¹ from Frasnes and 0.85 mg kg⁻¹ from Trazegnies). No exchangeable or soluble Cd was found. In the contaminated soils (Table 2) Cd reached high levels, being greatest in soils from Breinig (calcareous), but the mobility of the metal was much higher

Table 1 Localities and plant populations with their characteristics

Localities	Description of localities	Species collected and transplanted
Belgium, Plombières	Old Pb and Zn mine in eastern part of Province de Liège; soil very rich in Pb and Zn in form of ore and blast furnace scorias; vegetation consists of turf of <i>F. ovina</i> , <i>Armeria maritima</i> , <i>Viola calaminaria</i> ; soil pH 6.0–6.5	<i>Festuca ovina</i> and <i>Agrostis tenuis</i>
Belgium, Prayon	Industrial area near Liège characterised by aerial pollution consisting of Zn, Pb, Cd and SO ₂ ; old soils colonised only by <i>A. tenuis</i> and <i>Thlaspi coerulea</i> ssp. <i>calaminare</i> ; soil pH 4.5–5.5	<i>Agrostis tenuis</i>
Belgium, Trazegnies	Uncontaminated area near Charleroi; soil pH 6.0–6.5	<i>Agrostis tenuis</i>
Belgium, Frasneslez-Couvin	Uncontaminated area in Province de Namur; turf with <i>F. ovina</i> and <i>Bromus erectus</i> ; calcareous soil	<i>Festuca ovina</i>
Germany, Breinig	Near Aachen; calcareous hills with Zn and Pb ore; turf rich in <i>F. ovina</i> , <i>Bromus erectus</i> , <i>Armeria maritima</i> , <i>Viola calaminaria</i> , etc.	<i>Festuca ovina</i>
Germany, Mausbach	Near Aachen; sandy artificial hill formed by accumulation of the tailings of Zn and Pb ores; vegetation includes <i>Molinia caerulea</i> , <i>F. ovina</i> and <i>Armeria maritima</i>	<i>Festuca ovina</i>
Germany, Strempt	Near Aachen; sandy artificial wastes formed by accumulation of tailings of Zn and Pb ores; vegetation includes mainly <i>F. ovina</i> and <i>A. tenuis</i>	<i>Festuca ovina</i>

Table 2 Amounts of Cd, Zn and Pb in the contaminated soils of Plombières, Breinig, Mausbach, Strempt and Prayon

Site	pH	Organic matter %	Cd	Nitric acid extraction			Ammonium acetate extraction			Distilled water extraction		
				Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd
Plombières, from a well developed turf	6.3	17.2	31.0	29,087	9,126	14.3	3,327	1,682	1.0	54.8	0.8	—
Plombières, turf at an early stage of development	6.1	10.5	69.8	20,616	26,140	17.7	1,337	4,515	1.0	19.0	1.0	—
Breinig, from a well developed turf	7.5	14.0	144.0	41,275	10,269	27.3	1,631	568	0.0	7.6	—	—
Mausbach, from a turf at an early stage of development	7.2	9.5	59.1	14,850	6,675	13.8	927	494	0.0	5.6	—	—
Strempt, from a turf at an early stage of development	5.6	9.4	2.0	2,250	18,000	0.0	230	1,293	0.0	16.5	0.5	—
Prayon, well developed turf	6.3	—	135.0	5,300	1,100	19.5	427	70	0.0	—	—	—

Figures are given in mg per kg of dried soil at 105 °C; means of 20 samples.

2 yr in an experimental garden. Populations are described in Table 1. Soil samples corresponding to each population were analysed in three ways. (1) Total heavy metals were measured by extraction with concentrated nitric acid at 25 °C (10-g soil sample, dried at 105 °C with 25 ml of HNO₃ for 48 h). (2) Exchangeable cations were measured by extraction with 1 M NH₄COOH at pH 7. (3) Water-soluble elements were measured by extraction in distilled water at 25 °C (10-g soil sample, dried at 105 °C, shaken, in 25 ml of H₂O for 48 h). Plant samples (aerial green parts) collected at random in the different localities were dried at 105 °C and reduced to powder. Samples of 1 g of powder were digested with a mixture of nitric and perchloric acids. After filtration the samples were analysed by atomic absorption for Cd, Zn and Pb.

Heavy metal tolerance was determined by Wilkins's method, based on comparative root growth in toxic and

in the acid soils of Plombières. Smaller amounts of Cd were found in soils from Prayon. Soils from Strempt (Germany) were rich in Zn and Pb but no significant quantity of Cd was found.

Ernst¹⁰ considers that the amount of exchangeable metal is the maximum available to plants and that the soluble

Table 3 Amounts of Cd, Zn and Pb in plants from polluted areas

Localisation and species	Date of collection	Cd	Zn	Pb
Plombières, <i>A. tenuis</i>	5/74	1.0	372.0	129.0
Plombières, <i>F. ovina</i>	5/74	0.5	390.0	175.0
Breinig, <i>F. ovina</i>	6/74	1.6	179.0	43.0
Breinig, <i>Euphrasia stricta</i>	6/74	11.0	937.0	42.0
Breinig, <i>Hieracium pilosella</i>	6/74	22.0	483.0	34.0

Figures are given in mg per kg of dry weight.

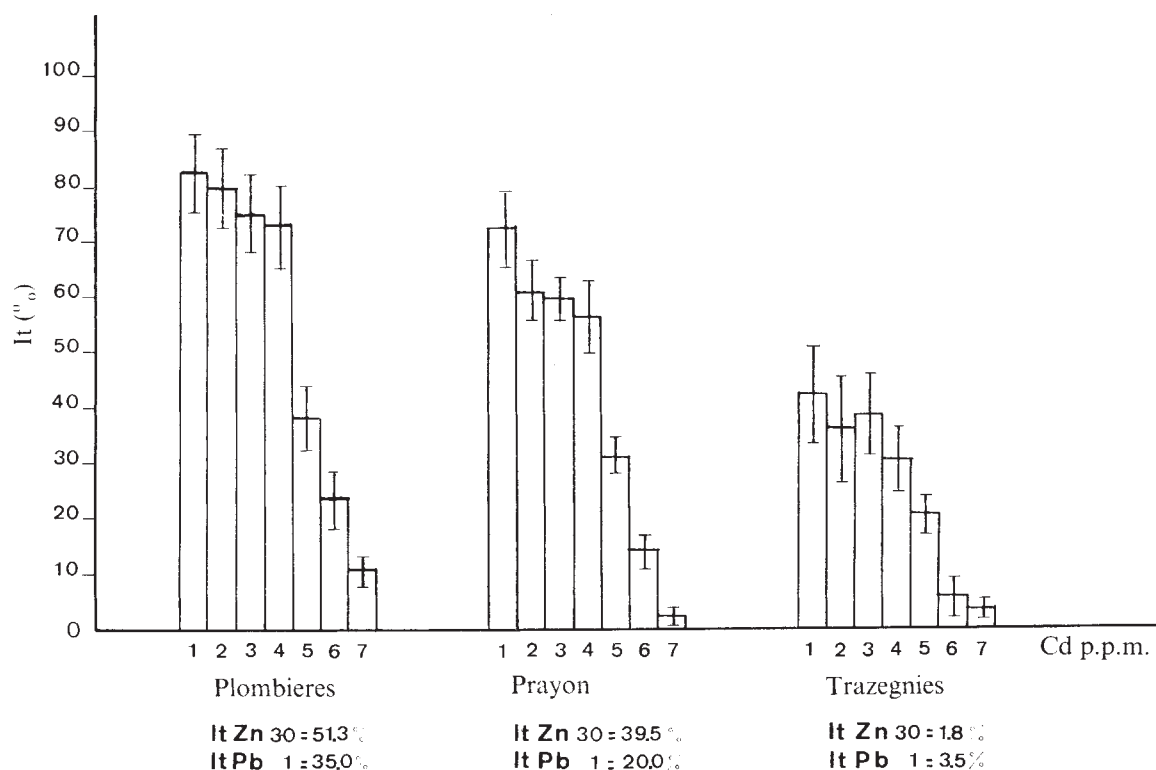


Fig. 1 Cd tolerance in *A. tenuis* populations at different concentrations of Cd. Plombières and Prayon are the contaminated areas. Trazegnies is the test population. Tolerance indices are given as percentages with standard errors. 1-7, Cd concentrations in the culture solutions: 1, 0.25 p.p.m.; 2, 0.50 p.p.m.; 3, 0.75 p.p.m.; 4, 1.00 p.p.m.; 5, 1.50 p.p.m.; 6, 2.00 p.p.m.; 7, 2.50 p.p.m. It Zn 30 is the tolerance index to Zn for the population grown on a solution with 30 p.p.m. of Zn. It Pb 1 is the tolerance index to Pb for the population grown on a solution with 1 p.p.m. of Pb.

fraction is the minimum available. Amounts of soluble Cd were very low compared with Zn (Table 2). My results for *A. tenuis*, *F. ovina* and some dicotyledons from Plombières and Breinig are shown in Table 3, together with values for Zn and Pb. Cd values were higher for dicotyledons than

for monocotyledons. *F. ovina* from Breinig (calcareous soil) contained more Cd than did the population from Plombières. The opposite pattern was found for Zn and Pb. This relates to the differential mobility of metals in relation to edaphic conditions.

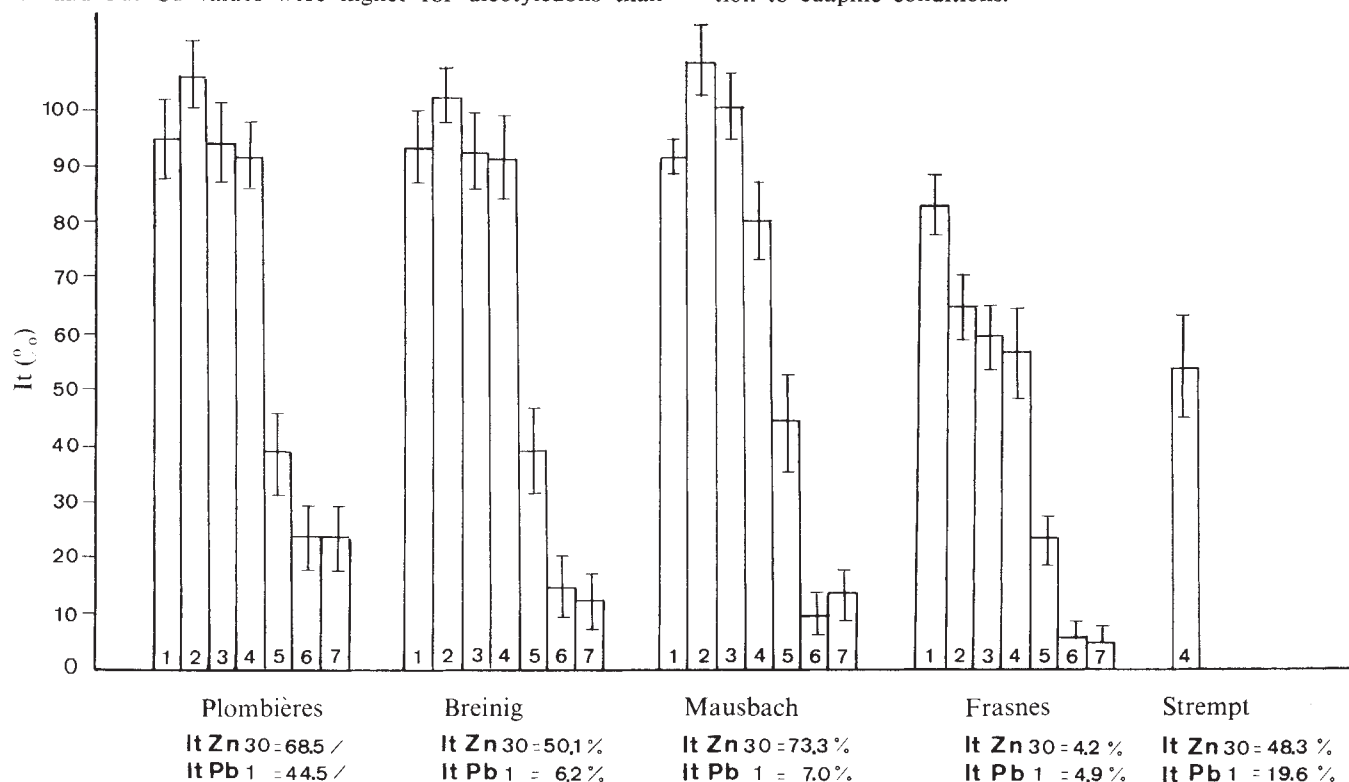


Fig. 2 Cd tolerance in populations of *F. ovina* at different concentrations of Cd. Plombières, Breinig and Mausbach are populations from contaminated areas. Frasnes and Strempt are the test population but Strempt is an area contaminated with Zn and Pb. The tolerance indices are given as percentages with standard errors. 1-7 are Cd concentrations in the culture solutions. 1, 0.25 p.p.m.; 2, 0.50 p.p.m.; 3, 0.75 p.p.m.; 4, 1.00 p.p.m.; 5, 1.50 p.p.m.; 6, 2.00 p.p.m.; 7, 2.50 p.p.m. It Zn 30 is the tolerance index to Zn for the population grown on a solution with 30 p.p.m. of Zn. It Pb 1 is the tolerance index to Pb for the population grown on a solution with 1 p.p.m. of Pb.

Table 4 Effect of some soil nutrients on the toxicity of Cd

Species	Populations	Cd 1 p.p.m.	Cd 1 p.p.m. + Ca 5×10^{-4} M	Cd 1 p.p.m. + Mg 5×10^{-4} M	Cd 1 p.p.m. + K 5×10^{-4} M	Cd 1 p.p.m. + Na 5×10^{-4} M
<i>A. tenuis</i>	Plombières	83.2 ± 6.9	97.8 ± 6.3	84.1 ± 6.9	118.6 ± 9.5	85.2 ± 6.6
	Prayon	62.3 ± 6.0	99.2 ± 6.2	70.6 ± 6.2	85.3 ± 9.6	58.2 ± 4.3
	Trazegnies	41.4 ± 6.1	106.6 ± 9.0	92.8 ± 9.2	96.3 ± 7.3	70.9 ± 6.3
<i>F. ovina</i>	Plombières	90.4 ± 5.3	96.5 ± 6.4	89.5 ± 6.3	99.4 ± 7.0	86.4 ± 6.0
	Breinig	89.7 ± 6.0	98.4 ± 6.0	91.4 ± 6.5	88.6 ± 6.8	87.5 ± 5.5
	Frasnes	56.5 ± 6.0	85.5 ± 5.7	89.7 ± 8.0	88.0 ± 6.7	60.2 ± 5.0

Tolerance indices are reported in % with standard errors. Plombières, Prayon and Breinig are the contaminated areas. Trazegnies and Frasnes-lez-Couvin are normal areas.

I next tested for Cd tolerance in populations of *A. tenuis* and *F. ovina* from the contaminated areas of Plombières, Prayon, Breinig, Mausbach and Strempt. As Figs 1 and 2 show, the populations of the contaminated areas were more tolerant than those from normal areas. Tolerance indices were very high for *A. tenuis* from Plombières and Prayon, but much lower for this species from the normal soil of Trazegnies. *A. Tenuis* populations from Plombières and Prayon are therefore genetically adapted to Cd toxicity. With *F. ovina*, high tolerance indices were found for the populations from Plombières, Breinig and Mausbach compared, with those for the population from the normal soil of Frasnes.

Thus my work on transplanted populations shows that Cd tolerance has a genetic basis. The population of Strempt was not tolerant to Cd even though it was tolerant to Zn and Pb (Fig. 2). In *A. tenuis* and *F. ovina* populations, however, a decrease in the tolerance index was generally observed at 1.50 p.p.m. Cd. At 2.50 p.p.m. (except the population from Plombières) the plants stopped growing. In addition, in Plombières *A. tenuis* was always less tolerant than *F. ovina* to the three heavy metals tested.

The effects of some soil nutrients were studied. *A. tenuis* and *F. ovina* populations from contaminated and normal soils were grown in a solution containing Cd (NO₃)₂ (1 p.p.m.) and different soil nutrients Ca(NO₃)₂, MgSO₄, K₂SO₄ and Na₂SO₄ each at 5×10^{-4} M. Table 4 shows that the addition of Ca, Mg or K to the Cd solution restored normal growth to the populations of both species, even when they had been collected from normal soils. Na did not reduce the toxicity of Cd. This effect has been observed for the same populations grown on Zn and Pb solutions⁸.

Cd has high mobility and is available to plants. In acidic soils solubilisation makes this availability greater than in basic soils. Plants from soils rich in Cd are genetically more tolerant to Cd than plants from normal soils. The significant differences in tolerance indexes between *F. ovina* and *A. tenuis* show that tolerance to Cd is a specific character. Apparently, cotolerance does not occur, for the *F. ovina* population from Strempt was not tolerant to Cd but it was tolerant to Zn and Pb.

Cd tolerance is probably not restricted to a few species of Poaceae and it would be interesting to test species from other families.

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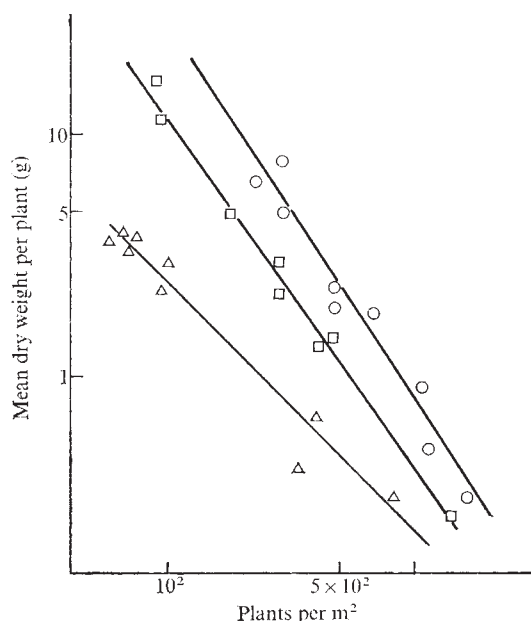
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Self-thinning driven by leaf area not by weight

THE self-thinning rule is perhaps the most widely applicable principle in plant population dynamics. White¹ has shown that it applies to more than 70 species ranging from herbs to trees, in many nutrient conditions. The rule states that $W = Kp^{-3/2}$, where W is average plant weight, p is the density of plants, and K is a constant. In other words when the log of average weight is plotted against the log of average density for a crowded even-aged plant population, numbers must be lost as the individuals gain weight in such a way that the population's trajectory is held under a line of slope $-3/2$.

Why the slope should be $-3/2$ has been unclear. The dimensional explanation has been put forward² that the weight of a plant increases as the cube of a size dimension, while the area occupied by it increases as the square of a

Fig. 1 Changes in density (p) and mean individual plant weight (W) of *Helianthus annuus* populations with time. The populations were grown at 100% (○), 60% (□) and 23% (△) light intensity. Data from starting densities of 1,600 and 400 plants per m⁻² are combined. Thinning lines shown are for 100% light $\log W = 3.84 - 1.33 \log p$, for 60% light $\log W = 3.53 - 1.30 \log p$, and for 23% light $\log W = 2.50 - 1.08 \log p$. After White and Harper², from data of Hiroi and Monsi⁴.



size dimension. But this reasoning does not explain the only significant exception to the $-3/2$ slope which has been found, which is that the slope is flatter under deep shade^{2,3}. This contribution shows that plants in low light develop more leaf area per unit weight, and that populations in both low and high light thin along the same slope in relation to leaf area.

I have found data where light levels were varied in studies both on self-thinning, and on the relation of leaf area to plant weight, only for *Helianthus annuus*. Self-thinning was studied by Hiroi and Monsi⁴ and analysed by White and Harper². The flattening of the slope of self-thinning at low light intensity is shown in Fig. 1. Blackman and Wilson⁵

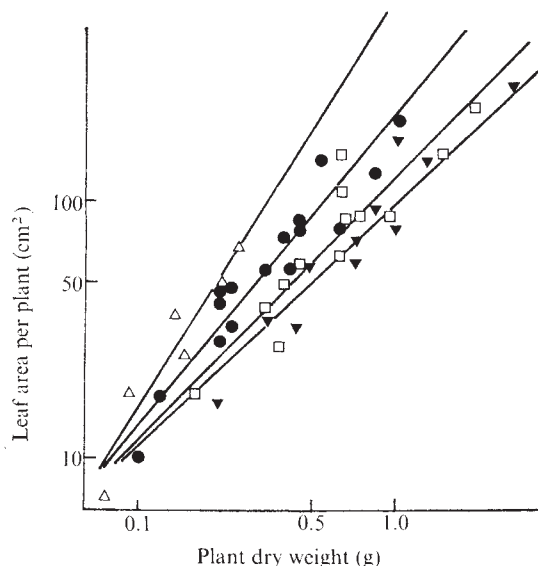


Fig. 2 Relation of leaf area per plant in cm^2 (L) to individual plant weight in g (W) at different light levels for *Helianthus annuus*, from data of Blackman and Wilson⁵. Data for six experiments are combined. Light intensities were 100% ($\blacktriangledown$), 50% ($\square$), 24% ($\bullet$) and 12% ($\triangle$) of full daylight. Best fit equations are, for 100% light, $\log L = 2.01 + 0.98 \log W$, for 50% light $\log L = 2.11 + 1.05 \log W$, for 24% light $\log L = 2.36 + 1.24 \log W$, and for 12% light $\log L = 2.83 + 1.63 \log W$. All equations have r^2 greater than 0.9. Where there were identical data points only one, from the lowest light level, is shown here, but all were used in calculating equations.

documented leaf area-plant weight relationships in different light intensities. Figure 2 shows that while leaf area per unit plant weight is constant at any one light level, it is greater in shade than in full light.

Both the flatter thinning slope, and the higher leaf area per unit weight, under low light, are common to all plant species studied^{3,5,6}, though *H. annuus* seems to be the only species for which both are documented.

The equations of Fig. 2 can be used to transform the data of Fig. 1 from a plot of weight against numbers to a plot of leaf area against numbers (Fig. 3). This completely removes the anomalous behaviour of the system at low light intensities. Thus it seems that the self-thinning rule in its most general form is $L = Kp^{-3/2}$.

Now the behaviour of plant populations while self-thinning is characterised (1) by the $-3/2$ slope just shown to be general if leaf area rather than weight is used as abscissa, (2) by the constant K , and (3) by the speed with which the population moves along the trajectory defined by $L = Kp^{-3/2}$, and the place where it stops. Formulating the self-thinning rule in terms of leaf area allows some further deductions on the third of these points. Since Lp is equal to the leaf area index (LAI) or area of leaf per unit ground area we can write $\text{LAI} = Kp^{-1/2}$. Thus we can relate the relative mortality rate ($-(dp/dt)/p$) of plants to the relative growth rate of the LAI ($(d\text{LAI}/dt)/\text{LAI}$); specifically $-(dp/dt)/p = 2((d\text{LAI}/dt)/\text{LAI})$. Thus the time dynamics

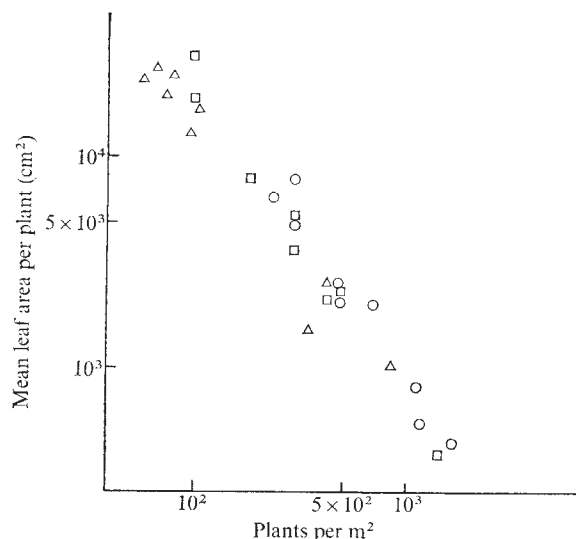


Fig. 3 Data on self-thinning in *Helianthus annuus* of Fig. 1 with average weight per plant transformed into average leaf area per plant using the equations appropriate to each light level from Fig. 2. Symbols for light intensities as in Fig. 1.

of mortality are driven by the function relating $d\text{LAI}/dt$ to LAI, a relationship which has been studied for several crops. In particular, we expect no further thinning when the canopy reaches maximum LAI⁶.

It is therefore to be hoped that future work on self-thinning will include measurements of leaf area as well as of weight and numbers of plants. As White and Harper² said when formulating the self-thinning rule: "It would be of great theoretical interest and of no little practical significance to determine the parameters of K for even a single species". Perhaps it will prove possible to explain K in terms of those canopy properties which determine optimal and maximum leaf area indices⁸.

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New structure associated with stomatal complex of the fern *Polypodium vulgare*

THE opening movements of stomata are generally considered to result from the guard cells gaining an osmotic advantage over their immediately adjacent epidermal cells resulting in the uptake of water into the guard cells and their consequent increase in volume. Fujino¹ and Fischer² independently proposed that active potassium transport between the guard and epidermal cells could be the mechanism by which the cationic fraction of the osmotic imbalance could be achieved. In recent years many investigators have produced corroborative evidence for this potassium shunt³ using Macallum's histochemical stain⁴. Further support for the hypothesis has been obtained using electron probe microanalysis⁵⁻⁸ and potassium-sensitive microelectrodes⁹. Despite these studies, no clear-cut picture has emerged concerning either the reservoirs of the ions at either end of the flux or the intercellular ionic pathway

although the findings of Heller *et al.*¹⁰ implicate the vacuole as the ion reservoir within the guard cells as do those of Penny and Bowling⁹. There is no current evidence of where or, indeed, whether, potassium is sequestered after it is shunted out of the guard cells during stomatal closure, but it has been suggested that the differentially thickened cell walls of the stomatal complex could serve as storage sites^{11,12}. We have recently discovered some new morphological features in the stomatal complex of the common polypody, *Polypodium vulgare* L., which we believe represent the site of potassium accumulation in this species.

Using the basic Macallum stain⁴ and viewing the stained epidermal strip from the side adjacent to the mesophyll

under a Zeiss Photomicroscope II, distinct polar accumulations of the ion can be observed in the guard-cell complexes (Fig. 1a). The accumulations are commonly up to 25 μm in diameter and can extend by as much as 5 μm beyond the guard cell walls at the poles (Fig. 1b). Associated with the guard cells, and especially with the polar accumulations, are potassium-rich strands that we construe as hollow trabeculae which bridge the common anticlinal walls of the guard and subsidiary cells. The polar accumulations show a distinct bimodal staining pattern which we interpret as indicative of (1) a darker, central circular site of ion adsorption and (2) a paler, bi-lobed osmotically/imbibitionally-induced swelling. This bimodal pattern is ill-defined in many guard-cell complexes because of the dense nature of the underlying chloroplasts but, interestingly, both elements of the polar structures are present in many dead guard cells which show no trace of protoplasts (Fig. 1c). Light microscopy studies suggest that the ion-adsorbent sites rest immediately on the outer surface of the guard-cells walls and that the osmotically/imbibitionally-induced sacs are located between the guard-cell walls and the endocuticle (Fig. 2).

Fresh material examined in an inverted position in the cryo unit of JSM-35 scanning electron microscope clearly shows that the polar accumulations observed with the histochemical stain are compatible with discrete substomatal sacs at the poles of the guard-cell complex and that the trabeculae are formed from extensions of the endocuticle immediately above the common sutures of the guard and subsidiary cells (Fig. 3a). The substomatal cuticular swellings are considered to represent the osmotically/imbibitionally-inflated products of the underlying ion-adsorbent sites determined with the histochemical stain. Epidermal strips fixed in 70% ethanol and observed with the scanning electron microscope indicate the collapse of the endocuticle on to the underlying cell walls and what we believe to be the actual ion-adsorbent areas underneath the substomatal sacs (Fig. 3b).

Using similar techniques, we have established the presence of substomatal sacs and ion-adsorbent areas in a wide range of filicopsid species but their absence in the psilopsid, lycopsid and sphenopsid pteridophytes we have been able to examine. What seem to be identical structures have also been found in some members of the monocotyledon family Commelinaceae, namely *Tradescantia virginiana*, *T. pallidus*, *Commelina communis*, *Callisia fragrans* and *Palisota barteri* (Fig. 4).

We can find no previous report of substomatal sacs in the literature although 'polar vesicles' were reported in the guard cells of *Casuarina*¹³ and analogous structures in the guard cells of *Psilotum*¹⁴. From these reports and our own studies on *Psilotum nudum*, it is clear that the polar vesicles are formed as a result of the dumb-bell-shaped lumen of the guard cells. Thickened common anticlinal guard-cell walls in *Vaccinium* spp. give rise to the appearance of polar pouches¹⁵ but a re-examination of this structure in *Vaccinium myrtillus* leaves us in little doubt that they are artefacts caused by the subsidiary cells entirely subtending the guard cells except in the polar regions. Differentially thickened common guard cell walls have also been reported by Raju *et al.*¹⁶. We confirm the existence of these specialised regions and have found them in many of the species we have examined (including *Polypodium vulgare*) but do not believe that they are directly connected with the actual substomatal sacs we report here.

The functional significance of these new structures is difficult to determine at present owing to the subdued responses of fern stomata in general. We have been unable to produce any clear evidence that stomatal movements in *P. vulgare* are anything more than extremely limited in extent although Lange *et al.*¹⁷ elicited responses to certain environmental conditions imposed on isolated epidermal

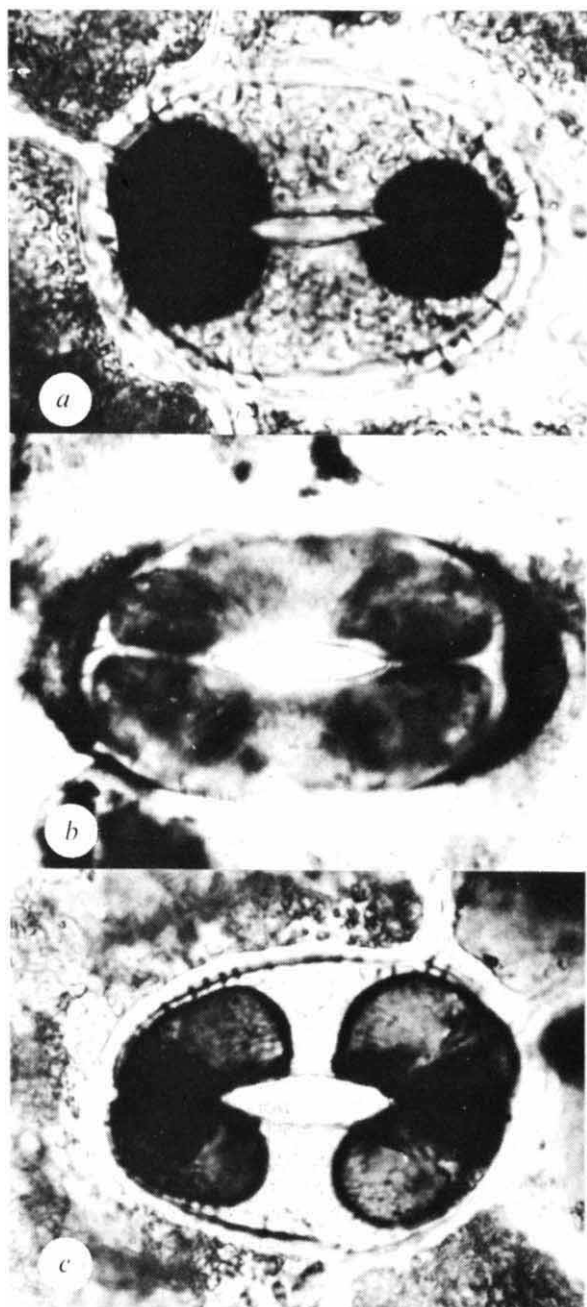
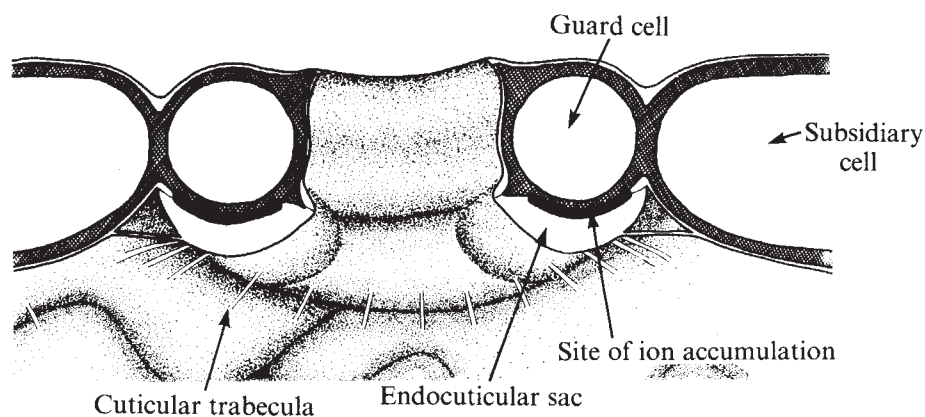


Fig. 1 Light micrographs of the stomatal complexes of *P. vulgare* stained for potassium using the Macallum technique, $\times 1,130$. *a*, Living complex viewed from the side adjacent to the mesophyll demonstrating the polar accumulation of the ion and the ion-rich trabeculae; *b*, living complex viewed from the outer surface demonstrating the polar accumulations subtending and extending beyond the polar cell walls of the guard cell complex; *c*, dead complex viewed from the side adjacent to the mesophyll demonstrating the two elements of the polar structures.

Fig. 2 Diagrammatic interpretation of a vertical longitudinal section through the polar structure in the guard cell complex of *P. vulgare*. The ion-adsorbent sites are represented by the solid black areas adjacent to the guard cell wall (cross-hatched) with the osmotically/imbibitionally swollen endocuticular sacs lying immediately below.



strips of this species. Meidner¹² records some inconsistencies between potassium content and stomatal aperture in the related fern, *Phyllitis scolopendrium*, a species which exhibits more typical stomatal responses¹⁸. We have obtained tentative evidence from scanning electron microscopy that the substomatal sacs become slightly smaller under dark conditions when compared with those under light conditions. Fern guard cells generally differ from those of typical angiosperms in having more numerous and larger chloro-

plasts which means that the vacuolar element of the protoplasts must be greatly reduced in volume. It could be that the substomatal sacs have been developed in *P. vulgare* and allied species to accommodate the accumulation of ions such as potassium in an extracellular position. If this is the case, it is difficult to see how the ions could have the necessary turgor effect on the guard cells required for stomatal movements. More probably, the function of the substomatal sacs is concerned with water conservation since cuticular water loss must be high in ferns because of the poor development of the cuticle in this group of plants. The inner faces of the guard and subsidiary cells are evaporative sites which make a major contribution to the water lost in

Fig. 3 Scanning electron micrographs of the stomatal complex of *P. vulgare* viewed from the side adjacent to the mesophyll, $\times 1,200$. *a*, Fresh material showing the polar substomatal sacs and trabeculae. A secondary signal distribution along the stomatal pore is superimposed on the micrograph to give an idea of the surface topography in this region. *b*, Material preserved in 70% ethanol showing the ion-adsorbent areas at the poles of the guard-cell complex.

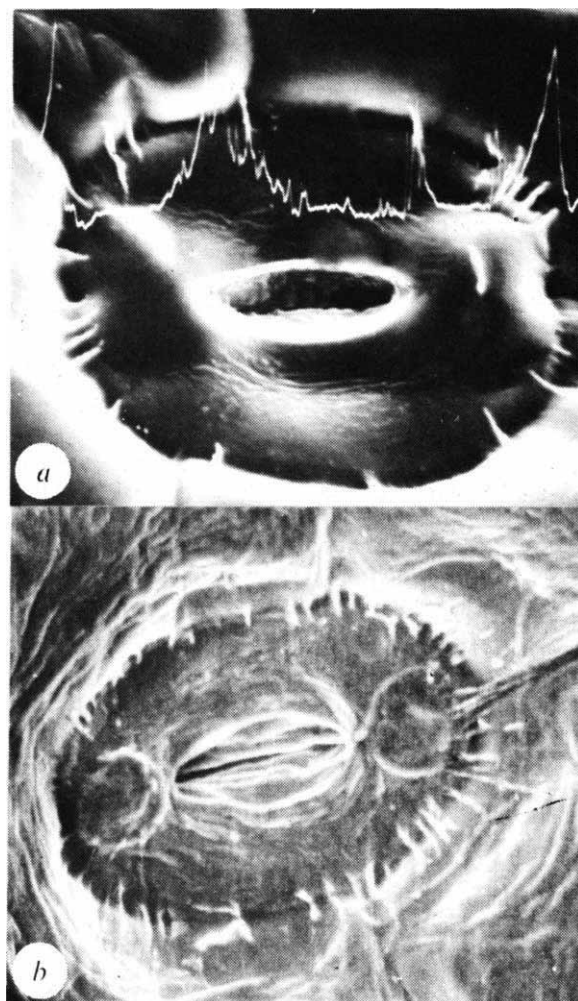


Fig. 4 Light micrographs of stomatal complexes viewed from the side adjacent to the mesophyll after staining for potassium using the Macallum technique. *a*, *Tradescantia virginiana*, $\times 790$; *b*, *Commelina communis*, $\times 1,010$.



stomatal transpiration¹⁹. The presence of potassium-rich sacs at these evaporative sites could reduce potential transpiration losses by virtue of their low osmotic potential and could also provide the guard-cell complex with a convenient source of cations for incorporation into the complex during stomatal opening.

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Stem anatomy and sheathing mycorrhizas in the *Betula verrucosa*-*Amanita muscaria* relationship

INCREASINGLY, attempts are being made to afforest difficult sites including prairie and steppe lands, peat bogs, *Calluna* heaths and industrial wastelands. In the United Kingdom the Forestry Commission is planting Sitka spruce (*Picea sitchensis* (Bong. Carr.) and Lodgepole pine (*Pinus contorta* Loud.) on land subject to periodic or protracted waterlogging and, notwithstanding the use of improved techniques of planting and site preparation, these species tend to be shallow rooted and liable to 'wind blow'. In these conditions the establishment of nursery-grown seedlings is problematical. As long ago as 1917, Melin² noted that while *Pinus sylvestris* L. and *Picea abies* (L.) Karst. transplants, used for afforesting freshly drained peat bogs, were without mycorrhizas they remained stunted. Growth accelerated as soon as these structures began to appear, however, presumably with the development of effective amounts of fungal inocula which may have been initially absent.

Until now the benefits accruing to hosts after mycorrhizal formation—increasing root and stem production³—have usually been attributed to enhanced mineral nutrition⁴. Marx and Bryan found that stems of trees with mycorrhizas grew taller and wider than those of trees without mycor-

rhizas⁵. In recent experiments, however, with (1) seedlings of one seedlot of birch (*Betula verrucosa* Ehrh.) and (2) two isolates of one of its ectomycorrhizal associates *Amanita muscaria* (L. ex Fr.) Hooker, it was found that different stem tissues were affected differently. Inoculating 10-d-old seedlings growing axenically on modified Ingstad's medium⁶ solidified with 1% agar, with isolates 1 and 2 taken from sporophores collected at Tomintoul and Penicuik, Scotland (Table 1) significantly increased stem diameters by 22 and 31%, respectively when assessed 8 weeks later.

Inoculation, which resulted in the formation of mycorrhizas within 4 weeks, increased widths of epidermal layers by 400 to 500% but those of vascular tissues by only 6 to 18%. But when transverse sections were examined microscopically, in addition to affecting the widths of differing bands of tissue, inoculation was found to have altered their nature (Table 2).

Table 2 Differences in stem anatomy of seedlings of *Betula verrucosa* with and without mycorrhizas formed with *Amanita muscaria*

Seedlings without mycorrhizas	Seedlings with mycorrhizas
Epidermis one cell thick without bark	Bark well developed, five cells thick
Lenticels absent	Lenticels developing
Cortex—small, thin-walled cells, closely adpressed, with very few intercellular spaces	Cortex—large cells with conspicuously 'thickened' walls, rounded with many distinct intercellular spaces
Phloem parenchyma—very infrequent occurrence of starch	Phloem parenchyma—most cells with starch and darkly staining substances
Xylem—secondary thickening patchily distributed	Xylem—uniform secondary thickening throughout tissue
Primary tissues—virtual absence of starch in pith	Primary tissues—most pith cells contain starch grains

Just as when pot-grown tomato plants were infected with the vesicular-arbuscular fungus *Glomus macrocarpus* var. *geosporus*⁷, so inoculation of *B. verrucosa* with *A. muscaria* was similarly associated (Fig. 1) with (1) the more uniform and probably thicker secondary thickening of the xylem, (2) larger and more rounded cortical cells with conspicuous intercellular spaces, (3) the relatively advanced development of bark, which was missing from non-mycorrhizal controls and (4) increased starch content of the phloem parenchyma and pith. Where it occurred, bark was punctuated with lenticels.

What advantages do these morphological changes, probably attributable to changed hormonal balances, confer on young trees, in view of the fact that they may reflect the ability of mycorrhizas to accelerate processes which might occur inevitably with increased age? They seem to be of two kinds related to (1) gaseous exchange, and (2) structural properties.

The effect of *A. muscaria* on stem cortex development can be regarded as an extension of a phenomenon identified by Coutts (see ref. 1, page 376). They found that air spaces

Table 1 Effects of inoculating roots of *Betula verrucosa* with the mycorrhizal *Amanita muscaria* on the width of stem tissues

Treatment	Width of stem tissues (µm)			
	Epidermis/bark	Cortex	Xylem/pith	Total
Uninoculated control	16	100	514	629
Inoculated with:				
isolate 1	100 (530)	121 (22)	543 (6)	765 (22)
isolate 2	85 (430)	138 (38)	604 (18)	827 (31)

Effects were assessed after 8 weeks of growth in axenic conditions at 26 °C with continuous light (mycorrhizas appeared 4 weeks after inoculation). Measurements were made on three sections of each replicate seedling. Figures in brackets refer to percentage increases.

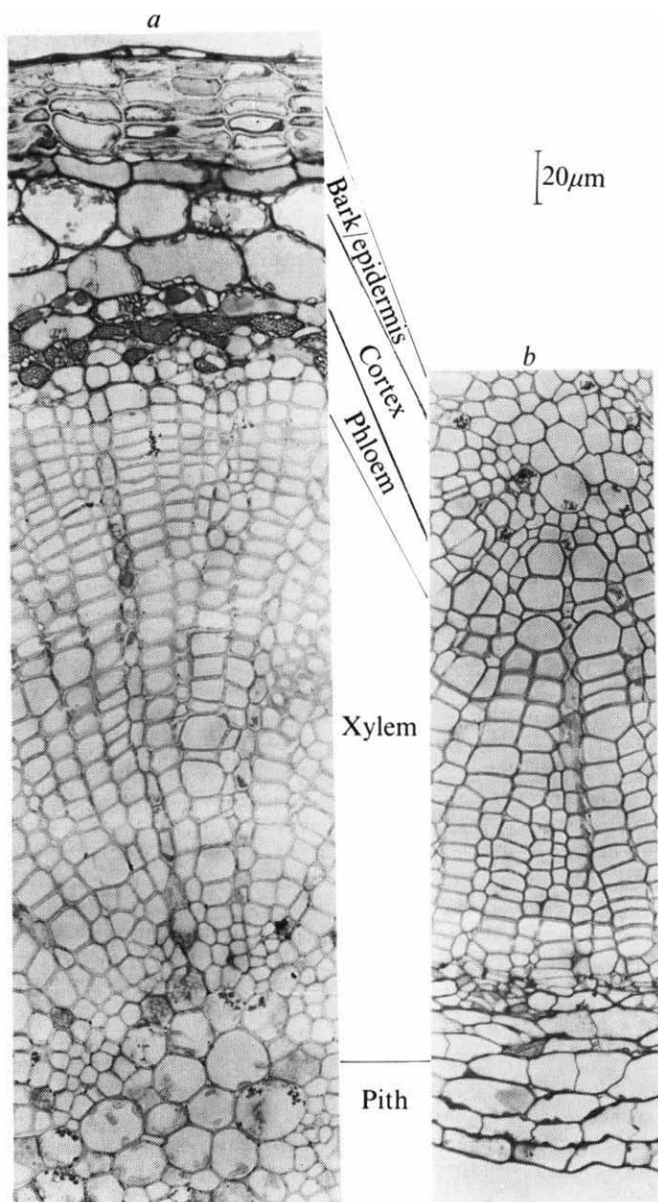


Fig. 1 Transverse sections of stems of *Betula verrucosa* cut 0.5 cm above cotyledons of seedlings *a*, with and *b*, without mycorrhizas formed with *Amanita muscaria*. (Material was fixed in buffered 3% glutaraldehyde and embedded in methacrylate before sections 4 μ m thick, cut with a glass knife, were stained with 0.5% toluidine blue.)

developed in the steles of roots of *P. contorta* growing in waterlogged and anaerobic conditions, the spaces possibly facilitating gaseous exchange through lenticels occurring on sections of root and stem. The accelerated appearance of bark and lenticels and the concomitant development of a spongier cortex in stems of *B. verrucosa* may serve a similar purpose, stem changes possibly complementing cortical alterations occurring in roots with sheathing mycorrhizas.

On the other hand, accelerated bark development might hasten the waterproofing of trees which otherwise might be at risk in arid conditions. Zerling, as mentioned in a review by Meyer⁸, found that sheathing mycorrhizas improved the water relations of trees in prairie and steppe regions.

Thus, while cortical changes in stems might facilitate tree establishment in 'stress conditions', whether these are associated with too much or too little water, the greater thickening of xylem elements and the early development of bark may confer structural benefits, not only increasing

quality (uniformity) and quantity of yield but also possibly aiding the ability of the tree to withstand exposure.

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Phytochrome-initiated fast rhythm controlling developmental response

To identify the steps controlling a developmental response, we must relate changes occurring soon after the inducing signal to responses many hours later. In higher plants exposure to red light shifts developmental patterns by converting phytochrome, a ubiquitous plant pigment, into its active form¹. Far red light converts phytochrome back to its original form and stops the response. Red light induces changes in electrical properties of plants in 15–30 s (refs 2, 3) and within minutes ATP levels⁴, NADP transport⁵, and nitrate reductase activity⁶ change. It requires hours or even days for phytochrome effects on circadian rhythms⁷, germination, de-aetiolation, and flowering responses to appear¹. Until now there has been no indication of the mechanism by which events immediately following phytochrome conversion determine the later development. It should be possible to probe the nature of the first controlling steps by perturbing these steps and thus affecting growth. If the steps directly following phytochrome conversion are sensitive to additional red light, then a second illumination will alter the overall growth. We have found that a brief flash of red light given to dark-grown bean seedlings starts oscillations in a temperature-compensated system. The interaction of this system with a second flash of red light showed that the oscillating system controls subsequent development.

Compared with unilluminated plants, bean seedlings (*Phaseolus vulgaris* var. Black Valentine) exposed to 10 min of white light and returned to dark have 40% greater leaf protein and leaf weight 24 h later⁸. Red illumination, 115 μ W cm^{-2} at 600 nm, saturated this growth response in 4 min, while our experiments used a total of 8 s of this red light. Given as a single flash, 8 s of red light increases average leaf weight by 8%. We used leaf weight increase in whole seedlings to measure the integrated phytochrome response.

To determine the effects of interference by a second red light on the reaction to the first, we kept total illumination constant and varied the interval between two 4-s flashes. For intervals ranging from 2 min 0 s to 2 min 30 s in increments of 5 s, we found that the length of the interval determined the leaf weight 24 h later. In 11 trials minimum growth occurred in the samples exposed to an interval around 2 min 15 s and maximum growth for an interval only 5 s longer. The maximum weight increase was 60% greater than the minimum. This sharp jump from low to

high weight increase is an easily observable feature to use in studying the interacting response to red light.

Given the repeatable difference in growth response to intervals of 2 min 15 s and 2 min 20 s, does the same minimum-maximum jump occur for any other pair of intervals 5 s apart? We extended the range of intervals from 0 min 5 s to 5 min 15 s and recorded average weight as a function of interval (Fig. 1). Arrows mark every significant minimum-maximum jump where probability from analysis of variance is less than 0.05. After the jump at 2 min 15 s to 2 min 20 s, the next significant one is at 3 min 0 s to 3 min 5 s, 45 s later. The likelihood of finding the next three jumps spaced at 45 ± 5 s intervals from these is only $3^3(3!24!)/27! = 0.009$. The part of the curve between the significant transitions shows hints of repetition but no outstanding features. Autocorrelation shows pronounced periodicity for 45 s, $P < 0.05$, and 90 s, $P < 0.01$. The 45-s periodicity persists at least 20 min. For intervals as long as 20–22 min, the minimum-maximum jump in the response appears at 20 min 20 s to 20 min 25 s and 45 s later at 21 min 5 s to 21 min 10 s.

Apparently red light shifts some system closely associated with controlling the growth rate into a rapidly oscillating state. If the changing sensitivity to a second illumination started by the initial red light is part of the phytochrome response, then far red light given immediately after the

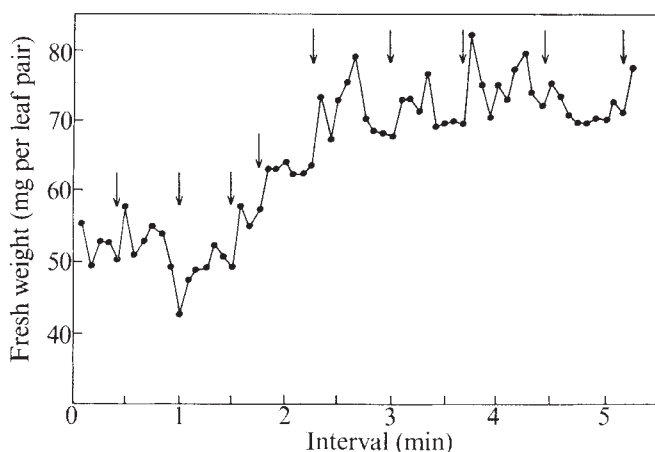


Fig. 1 The effect on leaf growth of the interval between two 4 s red illuminations. Average weight of primary leaf pairs from 8-d-old seedlings 24 h after red light. Arrows mark all the intervals where the second red light significantly suppresses growth compared to the interval 5 s longer, the minimum-maximum jumps. Plants were grown in the dark with standard nutrient: 5 mM $\text{Ca}(\text{NO}_3)_2$, 5 mM KNO_3 , 1 mM KH_2PO_4 , 1 mM MgSO_4 . Six pots of 15 seedlings each were given two 4 s red light pulses, $115 \mu\text{W cm}^{-2}$, with a measured dark interval between. The standard deviation among the samples for each interval ranges from 3 to 13%. For comparison, % std average fresh leaf weight: 7 d unilluminated, 9%; 8 d unilluminated, 5%; and 8 d with 10 min red light on day 7, 6%.

first red should eliminate the minimum-maximum jumps in response to the second red. There is no minimum-maximum jump at any interval when 4 s far red immediately follows the first red (Fig. 2). Far red light must either be damping the oscillations or desynchronising the plants so that the minimum-maximum jumps are no longer distinct. Variations among individual plants for any interval is no greater for initial red-far red than for red light alone, indicating that far red damps or stops the oscillations.

To prove that the rapidly oscillating system is phytochrome dependent, we must show repeated reversibility, with red light reversing the damping effect of far red. When red immediately follows far red it re-establishes the minimum-maximum jumps in response to the final red signal (Fig. 3). Arrows mark the significant jumps at

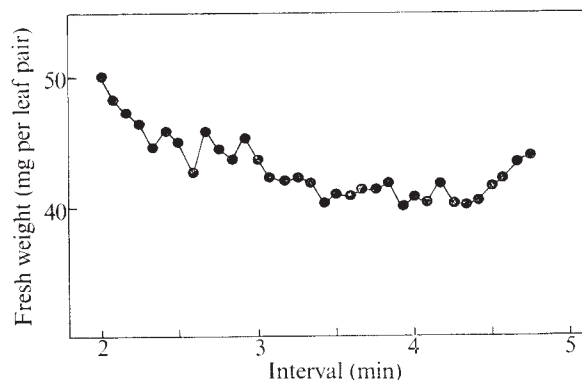


Fig. 2 Far red reversal. The effect on leaf growth of the interval between 4 s red light followed immediately by 4 s far red light, $2500 \mu\text{W cm}^{-2}$, and another 4 s red illumination after the dark interval. Other conditions as in Fig. 1.

2 min 5 s to 2 min 10 s and 3 min 15 s to 3 min 20 s in two replicate experiments. The shape of the curve is unlike the shape with red alone, but minimum-maximum jumps in the response are clearly not damped out as they are by far red light.

The period of the oscillation is independent of temperature between 15 and 30°C . The seedlings were grown, and experiments described previously were carried out at 22°C . To avoid the possibility that a temperature shift itself might produce time-dependent effects, we moved seedlings into the 15 or 30°C room at random times with respect to illumination. At 15°C , the entire plants equilibrated to 15°C . Minimum-maximum jumps in the response occurred 40 or 45 s apart between 3 min 0 s and 6 min 0 s. At 30°C , hypocotyls equilibrated to 30°C , but transpiration and evaporation lowered the leaf temperature to 25°C . For intervals ranging from 2 min 0 s to 4 min 0 s, there were three minimum-maximum jumps in the response, 45 and 40 s apart. The period of the oscillation is unchanged, but the phase of the oscillation is affected by the temperature during the illumination.

The results demonstrate that we can study the system controlling the phytochrome response by perturbing it with additional red light. The presence of oscillations shows that previous models⁹, which give the developmental response as a monotonically increasing function of the inducing red light, must be revised for subsaturating amounts of light. Initiation of these oscillations is among the first consequences of phytochrome conversion. The system is active

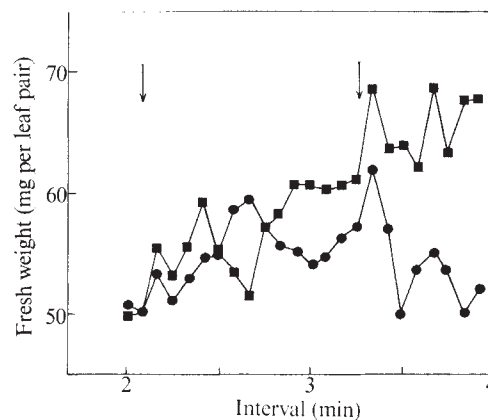


Fig. 3 Red reversal of far red. The effect on leaf growth of the interval between 4 s red light, 24 s far red, and 4 s red, followed by the dark interval and then another red illumination. Arrows mark the minimum-maximum jumps. Other conditions as in Fig. 1.

within 10 s, since growth responses to the second flash of red light at 5 and 10 s are significantly different, $P < 0.01$. It is certainly functioning within 25 s, even though it does not reach stable oscillations for 2 min. The temperature independence of the system implies that it is in some organised structure. The system must be linked to, but is probably not identical to, the phytochrome binding sites in the membranes. Since circadian rhythms in plants are sensitive to phytochrome and are temperature compensated, this oscillating system may be the mechanism by which red light interacts with those rhythms. The growth response to two red light signals shows that phytochrome conversion rapidly activates a new type of control linking early events to later development through an oscillating system.

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Two previously undescribed potato viruses from South America

THE proneness of potatoes (*Solanum* spp.) to virus diseases is well known, and about 20 viruses are recorded in naturally infected potatoes in Europe and North America^{1,2}. Many of these viruses also occur in the Andean region of South America. For example, in Peru several well known viruses were detected³ in 'basic seed' stocks by inoculating sap to a range of indicator species and by serological tests. Some indicator plants in these tests, however, developed symptoms unlike those typical of previously described potato viruses. These symptoms were of two main types, necrotic local lesions and systemic necrosis in *Chenopodium quinoa*, and systemic necrosis and shoot distortion in *Phaseolus vulgaris* cv. Pinto or Sanilac. We describe here the characterisation of the virus isolates producing these effects. Two previously undescribed viruses were identified: we call them potato black ringspot virus (PBRV) and potato virus T (PVT).

The stock culture of the first virus, PBRV, is an isolate obtained from a plant of the cultivar Antarkui (*S. tuberosum* subsp. *tuberosum* × *S. tuberosum* subsp. *andigena*) growing at Huasa Huasi, Junin, Peru and showing necrotic spotting on the tip leaves. In glasshouse conditions at Dundee, PBRV produces local lesions in inoculated *C. quinoa* leaves in 4 d and systemic necrosis in 6–8 d. Necrotic local lesions develop in 6 d in *P. vulgaris* cv. The Prince; systemic necrotic spotting is produced in 7–8 d and ultimately the growing point of the main shoot is killed. Potato plants (*S. tuberosum* subsp. *tuberosum* cv. Maris Bard) infected by inoculation of sap develop black rings and spots in systemically infected leaves. PBRV has a very wide host range, which will be described in detail elsewhere, occurs in moderate concentration in sap and is moderately stable (Table 1). Purified virus preparations, made from systemically infected *Nicotiana clelandii* leaves by the method of Murant *et al.*⁴; contain numerous isometric

Table 1 Some properties of PBRV and PVT

Properties in sap*	PBRV	PVT
Dilution endpoint	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}
Thermal inactivation point (10 min exposure)	58–60 °C	55–60 °C
Longevity at 20 °C	9–10 d	2–4 d
Particle shape and size (nm)	Isometric (25)	Flexuous filament (640 × 12)

*Source and assay plants respectively were *N. clelandii* and *C. amaranticolor* for PBRV, and *C. quinoa* and *P. vulgaris* cv. Pinto for PVT.

particles which measure about 25 nm in diameter in electron micrographs of samples negatively stained with phosphotungstate. Gel-diffusion serological tests indicate that PBRV is distantly related to tobacco ringspot virus (Table 2) and therefore belongs to the nepovirus group⁵, other members of which have soil-inhabiting nematodes as vectors and are transmitted through the true seed of many plant species.

Table 2 Serological relationship between PBRV and tobacco ringspot virus

Antiserum	PBRV	Antigen* Tobacco ringspot virus
PBRV	1/2048	1/32
Tobacco ringspot virus	1/16	1/512

*Figures are antiserum reaction end-points in gel-diffusion serological tests.

The stock culture of the second virus was also obtained from potato cv. Antarkui growing at Huasa Huasi, but the source plant showed no obvious symptoms of infection. This culture was passed through a series of local lesions, which develop in heavily shaded inoculated leaves of *P. vulgaris* cv. The Prince in 6 d; systemic vein necrosis is produced in 10 d, and leaves formed subsequently show a distorting mosaic. In *C. quinoa* PVT is much less virulent than PBRV, producing no symptoms in inoculated leaves and a mild systemic mottle after 10 d. Typically, no symptoms develop in potato cv. Maris Bard infected by inoculation of sap. PVT is less stable than PBRV in sap at 20 °C (Table 1) and has a narrower host range. Infective *C. quinoa* sap, negatively stained with uranyl acetate, contains flexuous filamentous particles measuring about 640 × 12 nm. Serological tests made by the tube precipitin method, using virus particles purified by a technique similar to that of Lister⁶, and antisera to purified virus, indicate that PVT is related to apple stem grooving virus (Table 3) but not to potato viruses S or M. Significantly PVT is frequently seed-transmitted in various solanaceous species; for example, of 18 progeny seedlings derived from experimentally infected *Datura stramonium* mother plants, 13 were infected.

Our report is intended to alert potato pathologists to the occurrence of PBRV and PVT, and to indicate how

Table 3 Serological relationship between PVT and apple stem grooving virus

Antiserum	PVT	Antigen* Apple stem grooving virus
PVT	1/256	1/64
Apple stem grooving virus	1/32	1/128

*Figures are antiserum reaction end-points in tube precipitin tests, after incubation for 5 h at 37 °C.

they can be detected. Both viruses seem to be widespread in Peru. Our findings also support the idea that the Andean region of South America can be considered the main centre of diversity not only of tuber-bearing species of *Solanum*⁷ but also of their viruses. Early export of infected potato tubers from South and Central America to various other countries is probably responsible for the present worldwide distribution of many potato viruses. Other viruses, which did not occur in the originally exported material or did not spread in the new country, are likely still to be confined to the Andean region. Indeed we have evidence of viruses in addition to PBRV and PVT that occur in South American potatoes but have not been reported elsewhere.

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Control of inner cell mass development in cultured mouse blastocysts

THE mouse blastocyst consists of a group of cells, the inner cell mass (ICM), attached to a small area of the inside surface of a sphere of trophectoderm cells. The trophectoderm gives rise to the extraembryonic regions of the conceptus while the ICM forms the embryo proper¹. At implantation trophoblast cells invade the uterine epithelium. Those overlying the ICM proliferate to form the ectoplacental cone and the extraembryonic ectoderm and the remainder undergo transformation into giant cells². The cells of the inner cell mass divide further, and, together with the extraembryonic ectoderm, give rise to a column of cells, the egg cylinder, that extends into the blastocyst cavity (blastocoele). Lactic dehydrogenase A subunits (4A, LDH-5) appear at this time³, and may be synthesised first in tissue deriving from the ICM⁴. Subsequently LDH-4 (3A1B) and LDH-3 (2A2B) appear as the contribution of B subunits to the pattern increases. If implantation is prevented, further development of the blastocyst is arrested⁵ and

LDH-5 fails to appear³. Implantation therefore seems to act as a trigger, not only for trophoblast proliferation, but also, directly or indirectly, for further development of ICM. In the work reported here we have varied the conditions of culture of isolated blastocysts and have elicited alternative forms of development that mimic those observed *in vivo* when implantation occurs or is prevented. We have shown further that the triggering of ICM development *in vitro* may represent the release from an inhibitory effect of trophectoderm.

Blastocysts flushed from the uteri of Q strain mice on the fourth day of pregnancy were cultured in supplemented Eagle's minimal essential medium with 20% foetal calf serum⁴ in various conditions intended to favour or inhibit attachment of the blastocysts to the substratum. The results obtained are summarised in Table 1 and illustrated in Fig. 1. On Falcon dishes, attachment and outgrowth begin asynchronously on the second day of culture and all blastocysts are outgrown by the third day, showing giant cell mass grew vigorously. Such clumps of blastocysts were neighbouring trophoblast cells. As shown in Table 1 (group A) such outgrowths synthesise substantial amounts of LDH-5. Groups B–D give the results obtained with three different suspension culture conditions intended to inhibit blastocyst attachment. These were the use of substrata unfavourable to blastocyst attachment and outgrowth (bacteriological or agar-coated dishes), and hanging drop culture of single embryos, either on plastic cover slips suspended in oil, or in inverted Falcon microtest plates with the wells filled to capacity (15 μ l) with medium. In each case synthesis of LDH-5 is greatly reduced. None of these conditions was completely reliable in preventing attachment during 3 d of culture. Blastocysts did eventually attach to the bacteriological dish or agar surface, and, in hanging cultures, to the oil–medium interface or to the air–medium interface. In all cases such attachment led to the disappearance of the blastocoele cavity and, rarely, to some increase in the mass of cells. Such 'collapsed' blastocysts often contained some LDH-5, presumably depending on the time of blastocyst 'attachment' and/or collapse relative to the time of collection. Attachment to the agar surface was undoubtedly responsible for the three batches of embryos in group B (Table 1) that synthesised significant amounts of LDH-5. Even more strikingly, if groups of blastocysts were cultured together in a hanging drop, they always coalesced rapidly, their blastocoele cavities disappeared and the total cell transformation and proliferation of the ICM and invariably found to be positive for LDH-5 (gel 4, Fig. 1). When blastocysts that had remained in suspension for 3 d were transferred to Falcon dishes, attachment and outgrowth began within 15 min in a very synchronous fashion and significant amounts of LDH-5 were synthesised by the following day (gel 6, Fig. 1).

Since these results suggested that further development is related in some way to a collapse of the trophectoderm, we

Table 1 LDH-5 synthesis in blastocysts and ICMs after 3 or 4 d of culture in different conditions

Group	Culture conditions	No. of batches of embryos*	No. of batches with LDH-5 band				
			—	+	++	+++	++++
A	Blastocysts (outgrowths) on Falcon dishes	16	0	0	0	5	11
B	Blastocysts (suspended) on agar-coated dishes	9	6	2†	0	1†	0
C	on bacteriological dishes	2	1	0	1	0	0
D	singly, in hanging drops	11	7	4	0	0	0
E	ICMs (suspended) on bacteriological dishes	2	0	0	0	0	2
F	singly, in hanging drops	2	0	0	1‡	0	1

* An average of eight embryos per batch was applied on each microgel.

† Attached.

‡ Two ICMs only.

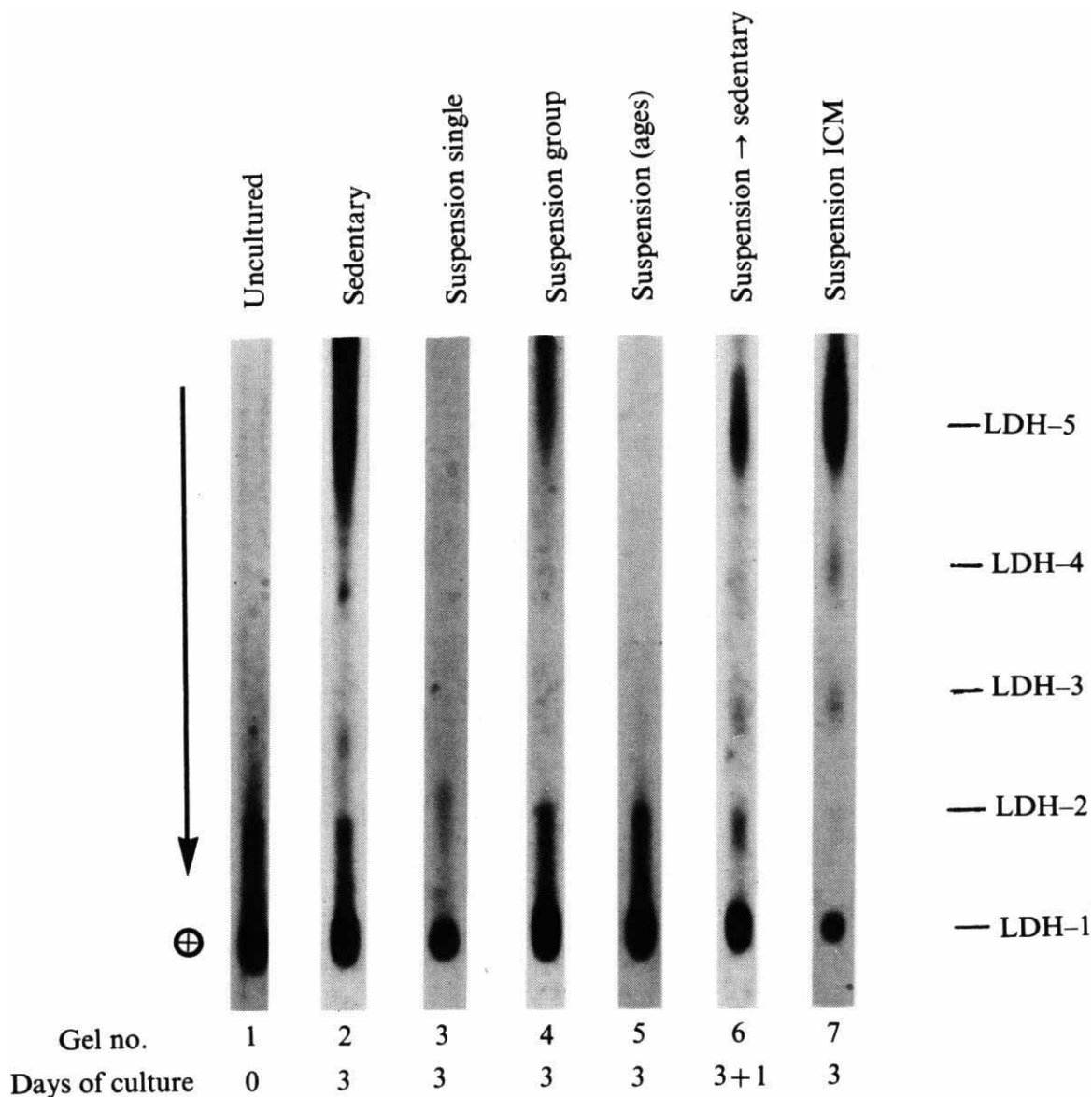


Fig. 1 Microdisk electrophoresis showing LDH isozymes in extracts of embryos after sedentary and suspension culture from the blastocyst stage. Isolation of blastocysts on the fourth day of pregnancy, media, and LDH staining were as described before⁴. Zonae pellucidae were removed before culture by 5 min of incubation in pronase. Polyacrylamide gel electrophoresis in 5- μ l Drummond caps was performed as described before⁷. Approximately 10 embryos were applied to each gel as follows: (1) uncultured blastocysts, (2) outgrowths from blastocysts after 3 d of sedentary culture on a Falcon dish, (3) blastocysts after 3 d of suspension culture singly in hanging drop, (4) blastocysts after 3 d of suspension as a group in hanging drop, (5) blastocysts after 3 d of suspension culture on an agar-coated dish, (6) outgrowths from blastocysts cultured for 3 d on an agar-coated dish followed by 1 d of culture on a Falcon dish, (7) inner cell masses after 3 d of culture in suspension on a bacteriological dish. All extracts contain residual maternally-inherited LDH-1 (ref. 4).

analysed isolated ICMs in suspension culture. Trophectoderm was removed immunosurgically⁶ by treatment of zona-free blastocysts with rabbit anti-mouse serum followed by guinea pig complement. Trophectoderm cells are lysed; ICMs, because they are not directly exposed to the anti-mouse serum, survive. Such ICMs grow and develop in suspension culture, forming a layer of primary endoderm cells on their surface after 2 d. In contrast to whole blastocysts, they synthesise LDH-5 in the absence of attachment and outgrowth (Fig. 1 and Table 1, groups E and F). This result suggests that implantation relieves an inhibitory effect of trophoctoderm on ICM development. This inhibition could be due to a restriction on free access for some nutrient(s), or to a more subtle contact effect.

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β -retinoic acid inhibits and reverses testosterone-induced hyperplasia in mouse prostate organ cultures

CERTAIN human prostatic cancers are associated with abnormalities of testosterone metabolism and/or increased sensitivity of prostatic epithelial cells to testosterone^{1,2}. Most treatments, (oestrogen therapy, orchiectomy and radiation therapy) for these diseases are designed therefore to reduce the levels of testosterone or its active metabolites in the patients³. Recently, however, studies with

Table 1 Inhibition of testosterone (1.8×10^{-5} M)-induced hyperplasia (m.i. $\pm$ s.d.)^{*†} by different concentrations of β -retinoic acid (RA)

Cultures were simultaneously treated with testosterone (1.8×10^{-5} M) and different concentrations of RA from the beginning of the experiment					
Days after treatment	Control	Testosterone (1.8×10^{-5} M)	RA (1.7×10^{-5} M)	RA (3.3×10^{-6} M)	RA (6.6×10^{-7} M)
4	148 $\pm$ 59	647 $\pm$ 261 $P < 0.01$ ‡	147 $\pm$ 45 $P < 0.01$ §	148 $\pm$ 25 $P < 0.01$ §	389 $\pm$ 87
8	145 $\pm$ 68	637 $\pm$ 185 $P < 0.01$ ‡	218 $\pm$ 81 $P < 0.01$ §	340 $\pm$ 136	239 $\pm$ 28 $P < 0.05$ §

*The number of arrested metaphases per 10^5 cells during a 4-h period of colcemid treatment.

†Mean and s.d. of 6–8 explants per group.

‡Mitotic stimulation significant as compared to the corresponding control group.

§Mitotic inhibition significant as compared to the corresponding group that received only testosterone.

non-hormonal chemotherapeutic agents for the treatment of prostate cancer have been initiated^{3–5}. In mouse prostate organ cultures, we and Lasnitzki have shown that vitamin A and several synthetic analogues (retinoids) inhibit and reverse carcinogen-induced epithelial lesions^{6–8}. Since testosterone has been linked with human prostatic cancer and induces hyperplastic lesions of the prostatic epithelium *in vitro*^{2,9}, it prompted us to start investigating the effects of β -retinoic acid (RA) and other retinoids on testosterone-induced lesions in the mouse prostate system. Preliminary results presented in this report show that RA inhibited and reversed testosterone-induced lesions in mouse prostate organ cultures.

Ventral prostate from 8- to 10-week-old C3H mice were removed aseptically and teased into approximately 1.5×1.0 -mm explants. The explants were arranged on strips of lens paper and were placed on stainless steel grids. Six to eight explants were placed on each grid that were contained in 35 mm plastic Petri dishes. Petri dishes were filled up to the level of the grids with CMRL 1066 medium (~ 2.5 ml) supplemented with 10% horse serum, 3% chick embryo extract, 100 U ml⁻¹ each of penicillin and streptomycin and 25 μ g ml⁻¹ amphotericin B. The cultures were incubated at 37 °C in an atmosphere of 50% O₂+45 N₂+5 CO₂. Culture medium was renewed every 48 h.

Two series of experiments were carried out. In the first, explants were incubated simultaneously with testosterone (1.8×10^{-5} M) and different concentrations of RA to determine if the development of hyperplasia could be prevented. In the second, the reversal of hyperplasia was examined. In these experiments, explants were first allowed to develop hyperplasia by treatment with the testosterone for 8 days, after which time, these hyperplastic explants were simultaneously treated with testosterone and different concentrations of RA for an additional 96 h. The changes in rates of cell proliferation caused by testosterone or RA were determined by the colcemid metaphase arrest technique⁶. The explants were fixed in buffered formalin, processed for

histology, serially sectioned, and the mitotic index and standard deviation (m.i. $\pm$ s.d.) determined as previously described⁶. The significance of the difference between two groups was determined by Student's *t*-test, and a value of $P < 0.05$ was considered significant.

Since the mitotic indices in different groups of explants were variable, the data presented in each table represent one experiment. Table 1 shows that testosterone stimulated cellular proliferation in the epithelium of prostate at 4 or 8 days after treatment, but that this stimulatory effect was prevented in explants that were simultaneously treated with testosterone and RA. For instance, at 4 d after treatment, the m.i. in the control explants was 148 ± 59 , whereas the corresponding value in testosterone-treated explants was 647 ± 261 . The m.i. in explants simultaneously treated with testosterone and RA were 147 ± 45 , 148 ± 25 and 389 ± 87 at 1.7×10^{-5} M, 3.3×10^{-6} M, and 6.6×10^{-7} M, respectively. A similar mitotic inhibitory effect by RA was observed at 8 d.

The morphology of prostate explants incubated in control media for 8 d showed little alteration from the *in vivo* structure. The alveolar epithelium remained 1–2 cell layers thick and exhibited little mitotic activity. Occasionally some Periodic-Acid Schiff (PAS) positive secretory material was present in the alveolar lumen of the control explants. Treatment of explants with testosterone produced hypertrophy and hyperplasia of the alveolar epithelium. The epithelium of these explants was tall, contained many mitoses, and showed a significant increase in the PAS positive material in the alveolar lumen. When, however, explants were treated simultaneously with the testosterone and RA (1.7×10^{-5} M), the degree of hypertrophy and hyperplasia was significantly less than that of explants that received the hormone only.

Table 2 shows the data on the reversal of testosterone-induced hyperplasia by RA and indicates that testosterone stimulated proliferation in the prostatic epithelium at 8 d, and that the values of m.i. in the testosterone-treated

Table 2 Reversal of testosterone (1.8×10^{-5} M)-induced hyperplasia (m.i. $\pm$ s.d.)^{*†} by different concentrations of β -retinoic acid (RA)

Cultures were treated with testosterone (1.8×10^{-5} M) for 8 d, followed by simultaneous treatment with testosterone and different concentrations of RA						
Days after treatment	Control	Testosterone (1.8×10^{-5} M)	Hours after adding β -retinoic acid	Testosterone + RA (1.7×10^{-5} M)	Testosterone + RA (3.3×10^{-6} M)	Testosterone + RA (6.6×10^{-7} M)
8	64 $\pm$ 14	556 $\pm$ 146 $P < 0.01$ ‡				
10	107 $\pm$ 56	753 $\pm$ 127 $P < 0.01$ ‡	48	133 $\pm$ 59 $P < 0.01$ §	103 $\pm$ 45 $P < 0.01$ §	185 $\pm$ 48 $P < 0.01$ §
12	140 $\pm$ 53	602 $\pm$ 227 $P < 0.01$ ‡	96	95 $\pm$ 18 $P < 0.01$ §	125 $\pm$ 30 $P < 0.01$ §	164 $\pm$ 60 $P < 0.01$ §

*The number of arrested metaphases per 10^5 cells during a 4-h period of colcemid treatment.

†Mean and s.d. of 6–8 explants per group.

‡Mitotic stimulation significant as compared to the corresponding control group.

§Mitotic inhibition significant as compared to the corresponding group that received only testosterone.

explants remained significantly higher than the corresponding control values for at least 12 d. But when explants were first treated with testosterone for 8 d followed by simultaneous treatment with testosterone and RA at 1.7×10^{-5} M, 3.3×10^{-6} M, and 6.6×10^{-7} M for an additional 48 or 96 h, the hyperplasia was reversed.

The mechanism of inhibition and reversal by RA of testosterone-induced hyperplasia in the mouse prostate is not known. It has been shown that steroid hormones interact with their specific proteins in the target tissues. In testosterone-dependent tissues for instance, the prostatic gland of experimental animals¹⁰⁻¹² and humans¹³, the existence of proteins with a pronounced affinity for the hormone has been documented. Furthermore, for its biological activity, testosterone must be metabolised (mediated by the enzyme 5 α -reductase) to 5 α -dihydrotestosterone, the form that appears to be responsible for the stimulation of proliferation of the prostatic epithelium^{3,14-16}. Thus the inhibition of binding of testosterone and/or its metabolism to 5 α -dihydrotestosterone would result in its failure to cause stimulation of proliferation. Sandberg *et al.*³ suggested that oestrogens, which have a remarkable efficacy in alleviating human prostatic carcinomas, act by reducing the testosterone titres directly via the pituitary¹⁷⁻¹⁹ and/or indirectly by inhibiting the enzyme 5 α -reductase^{20,21}. Since the prostate organ culture model used in the present investigation does not involve the pituitary, the inhibition of testosterone-induced hyperplasia by RA may be accomplished by inhibition of the binding of testosterone to the specific protein and/or by inhibition of the metabolism of testosterone. However, the anti-hyperplastic activity of RA can also be explained by several other mechanisms.

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Table 1 Virulence* of *E. histolytica* HK-9H₂ to hamster liver

Time since last passage (weeks)	Size of inoculum ($\times 10^5$ amoebae in 50 μ l)			
	0.5	2.5	4.5	8
4	7	49	—	—
11	—	—	—	100
17	0	8	—	—
34	—	—	8	18

*Virulence is expressed as the average extent of abscess as a percentage of the infected central liver lobe. Between five and 10 hamsters were used in each experiment. They were killed 1 week after infection.

medium⁵ for many years had lost their pathogenicity entirely. When very large inocula were used, however, (10^7 amoebae), a granulomatous lesion was evoked in the hamster liver^{6,7}. Our experiments with strains made freshly axenic, isolated from patients with amoebic dysentery, suggested that the number of amoebae required to induce a certain extent of pathology in the hamster liver increased with the number of transfers in axenic culture⁸. After a long period of axenic growth, strains probably maintain a very low degree of pathogenicity. Using two such strains, HK-9 and HB-301, we obtained results which may contribute to the understanding of the pathogenicity of *E. histolytica*. We were able to restore the virulence of the HK-9 strain with cholesterol and establish a higher degree of virulence by two successive passages through hamster liver (HK-9H₂). *In vitro* toxicity towards guinea pig leukocytes, and agglutinability induced by concanavalin A (con A) were markedly greater in strain HK-9H₂ than in HK-9. Antigenic analysis revealed a pattern for HK-9H₂ different from that of the HK-9 strain.

Amoebae were injected, after laparotomy of the hamster under anaesthesia with ether, through a 20-gauge needle. The volume of the inoculum chosen was as small as possible to avoid damage of the liver parenchyma. Virulence studies are detailed in Tables 1 and 2. On intrahepatic injection of 10^7 amoebae in 150- μ l-inocula, strain HK-9 showed no virulence, whereas HB-301 caused small abscesses occasionally. Two transfers on TP-S-1 medium to which cholesterol (0.35 mg per tube) was added increased the virulence of the HK-9 strain. Inocula of 5×10^6 amoebae induced small granulomatous lesions from which amoebae could sometimes be cultured axenically. The virulence of the resulting HK-9H₁ strain, that is HK-9 after one passage through hamster liver, was markedly increased. Further passages through hamster liver increased virulence even more, so that inocula as low as 5×10^4 sometimes caused abscesses. After 16 weeks virulence was reduced, however, with larger inocula required to induce lesions (Table 1).

Further experiments confirmed the effect of cholesterol on the virulence of the HK-9 strain (Table 2). Cholesterol did not further increase the virulence of HB-301 strain. Epicholesterol—the 3 α -hydroxy isomer of cholesterol which, unlike cholesterol, does not induce changes in the permeability of the cell⁹—had the same effect. Although amoebae were present in the small abscesses they could be cultured on TP-S-1 medium only twice; the number of amoebae was probably too small to initiate an axenic culture in other cases. When the hamsters were not killed after 1 week, but allowed to live on their normal diet of food pellets and water, the abscesses healed and eventually disappeared. An effect of cholesterol on the virulence of axenic amoebae has not been reported before. There is disagreement on the effect of cholesterol on the invasiveness of amoebae-bacteria cultures in the rat caecum¹⁰⁻¹¹.

Virulent HK-9H₂ and non-virulent HK-9 strains were characterised by at least three clear differences—toxicity for guinea pig leukocytes, con A-induced agglutination,

Virulence and toxicity of axenic *Entamoeba histolytica*

THE ability of cultured *Entamoeba histolytica* to cause ulcers in the caecum of guinea pigs and rats or abscesses in the liver of the Syrian hamster decreases rapidly on elimination of the accompanying bacterial flora¹⁻⁴. It seemed that some strains grown axenically on TP-S-1

Table 2 Effect of cholesterol and epicholesterol on the virulence of *E. histolytica* HK-9 and HB-301

Strain	Treatment	No. of experiments	Size of inoculum ($\times 10^6$)*	No. of hamsters with abscesses per no. of hamsters inoculated	
HK-9	Untreated	3	1.1-4.8	0/12	(0%)
	Cholesterol	5	1.2-5.8	6/15	(40%)
	Epicholesterol	2	1.5-2.0	4/8	(50%)
HB-301	Untreated	2	1.7-3.6	4/6	(67%)
	Cholesterol	2	1.5	5/8	(63%)

Cholesterol and epicholesterol were dissolved in chloroform and evaporated in culture tubes (0.35 mg per tube) before adding the TP-S-1 medium.

*Amoebae in 75-150 μ l. The largest inoculum tested consisted of 75 μ l packed cell volume and 75 μ l TP-S-1 medium. One week after infection lesions were usually more or less confluent granulomatous necrotic areas. Amoebae were demonstrated by microscopy or culture of smears, or in histological cross-sections.

and antigenic composition. Amoebae from 2- or 3-d-old cultures were washed in physiological saline twice and a suspension of 3×10^6 amoebae per ml was prepared. Leukocytes were obtained by draining the peritoneal cavity of a guinea pig of about 300 g 16 h after the injection of 55 ml of physiological saline. The suspension contained approximately 10^7 leukocytes per ml, of which half were polymorphonuclear and half mononuclear leukocytes (monocytes, macrophages and lymphocytes). On mixing leukocyte and HK-9H₂ amoebae suspensions, 50% of the leukocytes were killed in about 15 min (Fig. 1), so that each amoebae killed an average of about 1.7 leukocytes. The increasingly smaller chance of an amoebae hitting an intact leukocyte may explain the shape of the curve. Induction of contact-lysis by *E. histolytica* in human leukocytes and cell cultures has been reported before^{4,12,13}. Amoebae of the HB-301 and HB-301H₂ strains showed the

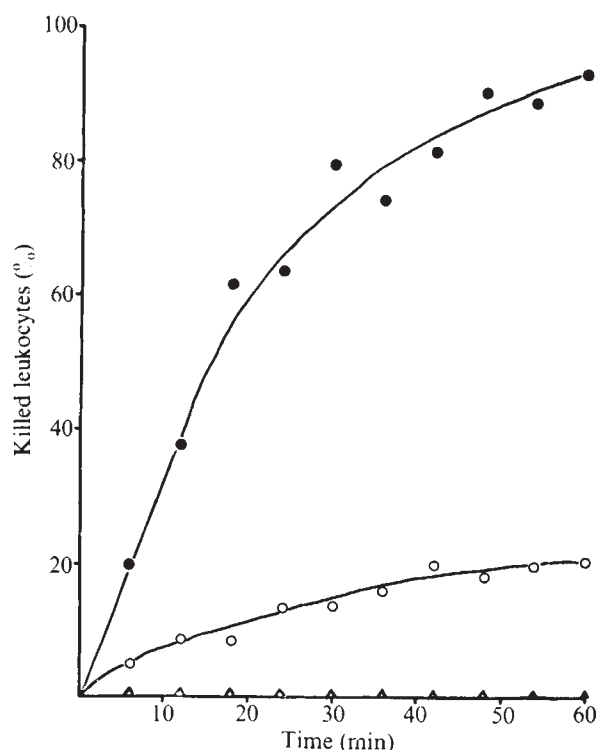
same degree of toxicity towards guinea pig leukocytes as HK-9H₂, whereas the original HK-9 strain seemed to have a very low toxicity. HK-9H₂ maintained its leukocytotoxicity on the same level even when its virulence decreased in the course of 34 weeks. Toxicity could not be increased in HK-9 amoebae by addition of cholesterol to the medium; at least one passage through hamster liver was required for restoration of toxicity. Interactions between amoebae and pure suspensions of guinea pig macrophages were also studied. Macrophages were separated from other cells in a peritoneal washout by using their adherence to glass. Macrophages seemed to be lysed by the amoebae just as quickly as the inflammatory exudate cells.

Martinez-Palomo *et al.*¹⁴ reported selective agglutination of pathogenic strains of *E. histolytica* induced by con A. We did not observe a clear correlation between con A-induced agglutination and virulence towards the hamster liver with our axenic strains. Only when HK-9 was compared with HK-9H₂ was a marked difference found. The technique of Martinez-Palomo *et al.* was modified, using microtitre plates and stepwise increase of con A concentration. Amoebae suspension (200 μ l) was put in the cups of the microtitre plate and 10- μ l drops of con A solution of 200 μ g ml⁻¹ were added one by one. After addition of a drop the suspension was shaken on a microshaker for 15 s and agglutination was assessed (0, +, ++, and +++) at a low magnification on the stage of an inverted microscope. Immediately after the second passage through hamster liver maximal agglutination was obtained in a suspension of HK-9H₂ amoebae of 10^6 ml⁻¹ with con A at 15 μ g ml⁻¹, whereas 40 μ g ml⁻¹ was necessary for HK-9. After 8 weeks *in vitro* cultivation the difference between HK-9H₂ and HK-9 was reduced to zero. Another passage through hamster liver restored the difference. HB-301 and HB-301H₂ both agglutinated at a con A concentration of approximately 20 μ g ml⁻¹.

Antigenicity was analysed by immunoelectrophoresis (IEP) using human and rabbit anti-*E. histolytica* sera, and gel chromatography on Ultrogel AcA 34 and AcA 44 (LKB). The IEP pattern of HK-9H₂ showed at least one more precipitation line on the cathodal side than that of HK-9. Gel chromatography revealed a greater absorbance at 280 nm in the fractions with a molecular weight between 150,000 and 400,000. This will be investigated further.

We conclude that an increase of virulence in an attenuated axenic strain is involved with some, more or less stable, characteristics, such as toxicity towards host cells, of the surface membrane and antigenic make-up. The stability of these characteristics may depend on the homogeneity of genotypes within the population of a strain, which are constantly submitted to selective forces. In particular, the degree of toxicity towards leukocytes seems to be a vital characteristic of a virulent strain. *In vitro* toxicity against guinea pig leukocytes provides a convenient, quantitative test, which may prove useful to assess the pathogenic potential of a strain. Treatment with

Fig. 1 Contact-lysis of guinea pig leukocytes by *E. histolytica* strain HK-9 (○) and HK-9H₂ (●). The control (△) was a suspension of leukocytes without amoebae. The ratio of leukocytes to amoebae was approximately 10:3. Samples of 15 μ l of each suspension were mixed on a clean microscope slide, covered with a coverslip and sealed with vaseline. The slide was maintained at 37 °C on a heated microscope stage and observed with a $\times 40$ phase contrast objective. The percentage of leukocytes killed (that is, showing rounding up, fusion of nuclear lobes and diminution of granules) was assessed every 6 min by counting 100 leukocytes at random. A vital stain was not used.



cholesterol or epicholesterol may be a useful way to adapt a non-virulent strain to hamster liver passage.

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Failure of BCG to protect calves against *Babesia divergens* infection

THE possible use of nonspecific immunity to control *Babesia* infections has not been much examined. Cattle treated with *Escherichia coli* lipopolysaccharide (1.25 µg per kg live weight) immediately before infection with *B. argentina* (= *B. bovis*) were slightly more resistant than controls¹. An interferon inducer, a double stranded RNA, slightly delayed death in mice infected with *B. rodhaini*². The question has been reopened by some work with *B. microti* and *B. rodhaini* in which mice injected intravenously (i.v.) with 2×10^7 *Mycobacterium bovis* strain BCG (Glaxo strain) became completely immune to intraperitoneal challenge with 10^6 *Babesia* parasites³. These remarkable results suggested that the method should be examined in domestic animals. We decided to assess it in calves and report here that BCG fails to protect against infection with *B. divergens*, the common cause of redwater in the UK.

In a preliminary trial two splenectomised Friesian bull calves were used; one was inoculated i.v. with 50 ml of BCG suspension (a fresh, wet preparation, Glaxo) containing an indicated 3 mg ml⁻¹. Thirty-four days later both calves were challenged by the i.v. inoculation of 0.1 ml of a *B. divergens* stabilate (Eire 2 strain). Both calves became infected and reacted similarly. This result was disappointing but because the use of splenectomised calves might have affected the outcome a second experiment using intact animals was carried out.

Glaxo provided 300 ampoules of lyophilised BCG vaccine, each ampoule containing 10^8 viable units. We dissolved the contents of each ampoule in approximately 0.5 ml of phosphate-buffered saline (PBS), the ampoules were thoroughly rinsed with PBS and the final suspension measured 200 ml. This was divided into three equal parts each of which contained approximately 10^{10} viable units. Each aliquot was inoculated immediately i.v. into a calf (K81, K82 and K84); no adverse side effects were noticed. Four weeks later these calves, together with three control animals (K56, K625 and K628) were challenged i.v. with 3.1×10^{10} parasitised erythrocytes (*B. divergens*, strain Eire 2). All the calves became infected; their reactions are

Table 1 Summary of results of challenge with *B. divergens*

Calf no.	Maximum parasitaemia (per 1,000 RBC)	Duration of parasitaemia (d)	Maximum temperature (°C)	Minimum RBC count ($\times 10^6$ mm ⁻³)
K81 : BCG	5	3	40.4	6.7
K82 : BCG	98	6	40.3	3.9
K84 : BCG	5	4	40.0	5.6
K56	5	3	39.7	7.1
K625	5	2	40.3	7.2
K628	3	3	40.1	6.1

summarised in Table 1. There was no indication that inoculation with BCG had suppressed the multiplication of *B. divergens*.

One of the BCG calves (K82) reacted noticeably more severely than the others. At necropsy no macroscopic abnormalities were noted in any of the calves.

Various reasons for this failure can be adduced: one possible explanation lies in the dose of BCG. We had calculated that to repeat the work in mice as closely as possible it would be necessary to give 10^{11} *M. bovis* organisms to each calf but it was clearly impracticable to administer more than 10^{10} organisms.

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Development of resistance to cytotoxicity during aflatoxin carcinogenesis

IT is now some time since Haddow first suggested that an early stage in chemical carcinogenesis might involve the induction of cells resistant to the toxic action of the carcinogen¹. Although it seems that many carcinogens also have acutely toxic properties, the relationship between these activities is largely unknown. We have investigated this relationship by using the mycotoxin aflatoxin B₁, and shown that resistance is linked with proliferation of specific hepatocytes.

Aflatoxin B₁ is a potent hepatotoxin and hepatocarcinogen in many animal species including the rat². Feeding young adult male Fischer rats a diet containing a low concentration (4-5 p.p.m.) of aflatoxin B₁ for 6 weeks, followed by return to control diet, results in 100% incidence of hepatocarcinoma³. During the first 3 weeks of the 6-week feeding regime, histological examination of the liver indicates an acutely toxic response, accompanied by depressed nucleic acid synthesis⁴. If toxic feeding is stopped at this stage an insignificant incidence of neoplasia results. The acutely toxic action results in the death of a considerable proportion of the hepatocytes at the end of the 3 weeks. The second 3 weeks of the 6-weeks feeding regime, during which the carcinogenic response is initiated, is accompanied by a proliferation of both parenchymal and non-parenchymal cells^{3,4}. Nucleic acid synthesis also recovers⁴. The cells which proliferate to replace those killed by the acutely toxic action of the first 3 weeks feeding do so in the presence of a continuing supply of the toxin, showing

that they possess a mechanism which makes them resistant to the acutely toxic, but not to the carcinogenic, actions of aflatoxin. The diet-induced resistance of the rats to the acute toxicity of aflatoxin is shown by the results in Table 1. The LD₅₀ is increased at least 2–3-fold. This increase in the LD₅₀ is paralleled histologically, by resistance to the hepatonecrotic effect of the toxin.

The possibility that the resistance of the liver cells in the whole animal to the acutely toxic action of aflatoxin B₁ could also be demonstrated *in vitro* was examined. Hepatocytes were isolated, and subsequently cultured, from livers of control animals (BL8) and livers of animals which had received the aflatoxin-contaminated diet (BL7) for 6 weeks. Morphologically, BL7 and BL8 cells appeared similar, but when treated with aflatoxin, BL7 cells survived and BL8 cells became structurally disorganised and detached from the dish (Fig. 1). Thus the resistance to the acutely toxic action of aflatoxin B₁, which was observed in the hepatocytes of intact animals which had received the toxic diet for 6 weeks, was also displayed by cells isolated from those livers and subsequently maintained in culture *in vitro* for 4 d. The isolated cells were not dividing at this time. In further experiments, cells isolated from control animals (BL8) or animals fed the toxic diet (BL7) were cultured with the medium being changed at approximately weekly intervals. After several weeks, or much sooner in cells isolated from rats fed the toxic diet, certain of the cells in the cultures started dividing, and numerous individual colonies were formed. Colonies were removed from the dish by trypsinisation and the dispersed cells were reseeded to establish rapidly dividing epithelial cell lines from each of the original maintenance cultures (BL7L and BL8L). These cell lines were indistinguishable morphologically. After several passages, the sensitivity of the cells to aflatoxin B₁ was again determined. Resistance of the original hepatocytes in the case of the aflatoxin-fed rats was still present in the cell lines derived from them. These results demonstrate that the resistance to the acutely toxic action of aflatoxin B₁, once established in the cells, is stable. Although most BL8L cells were killed when treated with aflatoxin B₁, a few survived. Dishes were washed with fresh medium 3 d after treatment with the toxin to remove dead cells, and returned to the incubator. Several weeks later colonies were observed to be growing from these surviving cells. These cells were then subcultured, after becoming confluent, and were subsequently passaged several times. Their response to the addition of aflatoxin B₁ to the medium was then determined. Cells derived from the susceptible cell line (BL8L) were now as resistant to the acutely toxic action of aflatoxin B₁ as the BL7L cells which

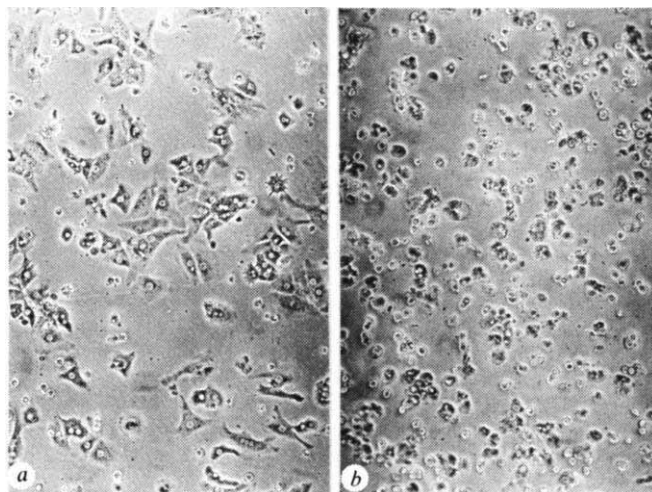


Fig. 1 Hepatocytes were isolated by perfusing liver excised from young adult male Fischer rats through the portal vein with collagenase solution (0.5% w/v) using a modification of the technique of Seglen⁶. The cellular suspension was further dissociated by agitation as described by Droockmans *et al.*⁸. Approximately 3×10^8 cells were obtained from each liver, with 80% viability assessed by exclusion of Trypan blue. Gross contamination was reduced by centrifugation twice at 50g for 2 min, and remaining cell clumps, broken cells and reticulo-endothelial cells were removed by sedimentation at 1g in a 1–4% Ficoll gradient at 4 °C in a modification of the apparatus described by Tulp and Bont⁷. This process also yielded final cultures free of fibroblasts. 8×10^7 cells were layered on to a gradient. After collection by low-speed centrifugation, cells were suspended in Williams E medium containing 10% foetal calf serum (Flow Laboratories, Irvine, Scotland) at 1×10^6 cells per ml and 3 ml was seeded per 60 mm plastic Petri dish. Medium was changed after 1 d. After 3 d, medium was again changed and cells were treated with aflatoxin B₁ (0.3 $\mu\text{g ml}^{-1}$ medium) in DMSO (6 $\mu\text{l ml}^{-1}$ medium). Control dishes received DMSO alone. Appearance of cells after 2 d treatment with aflatoxin: a, cells isolated from rat which had received the toxic diet (see Table 1) for 6 weeks (BL7); b, cells isolated from control-fed rat (BL8). Cells receiving DMSO alone appeared as in (a).

had survived treatment *in vivo*. It therefore seems that resistance to the acute toxicity of aflatoxin B₁ depends on the proliferation of certain hepatocytes with either an inherent resistance to aflatoxin toxicity or the capacity for the induction of that resistance by aflatoxin B₁, either in the whole animal or in culture.

The basis for the resistance is not known. It is not due to an overall failure of the cells to metabolise the toxin, for examination (thin-layer chromatography on silica gel G plates developed in chloroform-methanol, 9:1, (v/v), viewed under ultraviolet light) of a chloroform extract of the medium and cells, after incubating resistant cells (BL7L) in the presence of aflatoxin B₁ for 2 d, showed a considerable decrease in the concentration of residual aflatoxin B₁ compared with that found in control dishes which contained medium and aflatoxin B₁ but no cells. The resistance of the isolated cells and of the liver *in vivo* could be associated with a failure of the new cell population to metabolise aflatoxin B₁ specifically to an active metabolite. Such metabolism seems necessary for the inhibition of hepatic nucleic acid synthesis in *in vitro* systems⁸, which correlates with the toxic effect of aflatoxin *in vivo*⁴. The results given in Table 2, however, show that neither the capacity of the microsomes to activate aflatoxin B₁, nor the susceptibility of nuclear RNA polymerase to the active metabolite, is affected by feeding the toxic diet. Other possibilities are that the resistance could be associated with the rapid repair of nuclear lesions, or with detoxification by other cellular factors. Our observation that the reduced glutathione content of the liver doubles as a result of 6

Table 1 Effect of feeding a low level of aflatoxin B₁ on its acute toxicity

Aflatoxin B ₁ injected (mg kg ⁻¹)	Surviving after 7 d	
	Control diet	Toxic diet
1.5	0	3
1.25	0	3
1.0	0	3
0.75	0	3
0.5	3	3
0.25	3	3
0	3	3

Control diet: 50% non-toxic peanut meal/50% powdered MRC 41 B diet plus 3.3% Arachis oil. Toxic diet: 25% toxic peanut meal (naturally contaminated with 16 ppm aflatoxin B₁), 25% non-toxic peanut meal, 50% powdered MRC 41B diet, 3.3% Arachis oil. Diets were fed for 6 weeks to young adult male Fischer rats (150–200 g body weight). Groups of three animals were injected with aflatoxin B₁ dissolved in dimethylsulphoxide (DMSO), all animals receiving a total injection volume of 200 μl . All deaths occurred within 3 d of the injections. No further deaths occurred until 3 weeks from time of injection, when the experiment was terminated.

Table 2 Activation of aflatoxin B₁ *in vitro* to a form inhibitory to RNA polymerase *in vitro*

Source of fraction			RNA polymerase activity (d.p.m. incorporated per mg DNA)
Nuclei	Microsomes	NADPH	
CON	CON	—	55,500
CON	CON	+	19,200
CON	TOX	+	19,500
TOX	TOX	—	52,800
TOX	TOX	+	21,700
TOX	CON	+	19,700

TOX, animals fed toxic diet for 6 weeks (see Table 1); CON, animals fed control diet for 6 weeks. Assays carried out as described by Godoy and Neal⁸. Fractions were used in two successive incubations. First incubation, for activation of aflatoxin B₁: medium contained 50 μ M KCl, 150 μ M Tris-HCl pH 7.4, 12.5 μ M MgCl₂, 20 μ l DMSO containing 10 μ g aflatoxin B₁, 0.4 ml microsomal suspension (3.2 mg microsomal protein in 0.15 M KCl) and 1 ml nuclear suspension containing 150–200 μ g DNA (in 0.44 M sucrose/1 mM MgCl₂), 0.75 μ M NADP, 10 μ M glucose-6-phosphate and 0.5U glucose-6-phosphate dehydrogenase were added to incubations as indicated. After 10 min incubation at 37 °C in air, nuclei were spun down at 4 °C and supernatant was decanted. Second incubation: Mg²⁺-primed RNA polymerase activity of nuclei was determined as incorporation of label from ³H-UTP during 10 min at 37 °C. Results are means of triplicate assays, variation < $\pm$ 10% of mean.

weeks of feeding with aflatoxin (unpublished) could be relevant to the detoxification process.

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H-2 compatibility requirements for T suppressor cell functions induced by Friend leukaemia virus

GENE products of the major histocompatibility complex (H-2 in the mouse) regulate cellular interactions between various immunocompetent cell types^{1–4} as well as interactions between thymus-dependent (T) killer cells and chemically altered or virus-infected target cells^{5,6}. Identity of certain determinants of H-2 seems to be necessary for effective interaction between any two participating cell types. We have investigated the H-2 compatibility requirement for the suppressive interaction between T suppressor cells and mitogen-responsive cells in cultures infected with Friend leukaemia virus and find that there is an H-2D compatibility requirement. However, the H-2 restriction we observe is mediated by a third cell type which we term an 'interfering cell'. The two principal cell types, that is, T suppressor cells and mitogen-responsive cells, can interact even if they do not share any H-2 determinants provided that the interfering cells are functionally absent. This may provide an alternative explanation for the role the major histocompatibility complex in regulating other immune cellular interactions.

Friend leukaemia virus complex (FV) induces erythro-

leukaemia and causes severe immunosuppression in genetically susceptible adult mice⁷. FV mediates immunosuppression, at least in an *in vitro* model, by activating T suppressor cells^{8,9}. The T suppressor cells prevent T and bursa equivalent-dependent (B) cells from responding to a variety of mitogens in the presence of FV. The population size and/or function of these T suppressor cells in the body are under the regulatory influence of marrow-dependent (M) cells, since treatment of genetically resistant C57BL/6 mice with ⁹⁰Sr to destroy M cells induces the generation or appearance of T suppressor cells. T suppressor cells are also present in thymuses of infant C57BL/6 mice before the maturation of M cells. Mixtures of spleen cells from normal C57BL/6 mice, which have no detectable T suppressor cells, with spleen cells from ⁹⁰Sr-treated C57BL/6 mice are suppressed by FV *in vitro*⁸. We have taken advantage of such a mixture protocol to determine the H-2 compatibility requirements between T suppressor cells and mitogen-responsive cells.

Spleen cell suspensions containing mitogen-responsive cells (but not suppressor cells) from 'resistant' B10.D2, B10.A, and C57BL/6 mice responded well to the mitogen, concanavalin A, in the presence of FV (Table 1). Addition of small numbers (10⁵) of H-2-identical or semi-allogeneic 'susceptible' spleen cells, containing T suppressor cells, to the 9 × 10⁵ spleen cells of 'resistant' mice resulted in suppression of mitogenesis by FV. The suppressor cells functioned after exposure to 800 R of γ rays *in vitro*.

However, suppression across the H-2 barrier was not effective. Adult DBA/2 (H-2^d) spleen, marrow or thymus cells failed to suppress C57BL/6 (H-2^b) spleen cells. The data of Table 1 and Fig. 1 clearly indicate that donors of cell suspensions containing T suppressor cells and the mitogen-responsive cells must share the same H-2D determinant for FV to induce immunosuppression *in vitro*. The critical experiment was the differential ability of 129 and DBA/2 spleen cells to suppress HTG lymphocytes (Fig. 1).

One explanation for the H-2 compatibility requirements for cytolytic T cells and target cells has been that T cells react to 'altered self'^{10,11}. Indeed, antibodies directed against H-2D determinants inhibited cytotoxicity of syngeneic tumours by sensitised T cells^{10,11}, and an H-2D compatibility requirement for cytotoxic T cells against Friend virus-induced tumours was observed¹². An H-2D-end compatibility requirement for T suppressor cells has also been previously described¹³. In the AKR leukaemia model, T suppressor cells were restricted both by H-2 and non-H-2 differences because of an 'allogeneic effect'¹⁴. Another explanation for H-2 restriction has been the 'clonal selection' of T cells reactive to antigens presented on cells of a given H-2 type¹⁵.

The H-2 compatibility requirement does not exist for

Fig. 1 H-2D compatibility requirements for mitogen-responsive cell-T suppressor cell interaction. See Table 1 for details of protocol. Open squares are H-2^d determinants; solid squares are H-2^b determinants; hatched squares are H-2^k determinants.

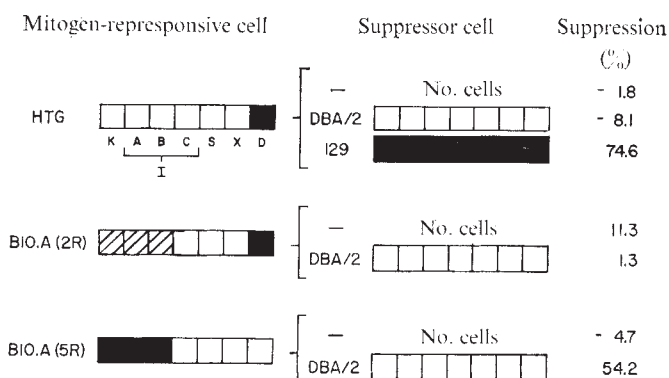


Table 1 H-2 compatibility requirements for Friend virus-induced suppression of mitogen-responsive cells by T suppressor cells

Experiment	Donor of spleen cell population		Change in Blastogenesis [‡]		Suppression %
	Resistant* (Mitogen-responsive cells)	Susceptible* (T suppressor cells)	-FV [†]	+FV	
1	B10.D2 (dddddd)§	None	62,573 ± 2,699	62,051 ± 2,714	0.8
		DBA/2 (dddddd)	60,234 ± 882	28,138 ± 1,713	53
		129 (bbbbbb)	49,314 ± 4,115	48,590 ± 497	1.4
		C3H (kkkkkk)	54,902 ± 692	57,863 ± 3,723	-5.0
2	B10.A (kkkkdd)	None	49,971 ± 2,708	45,048 ± 1,581	9.8
		A (kkkkdd)	50,150 ± 2,885	11,844 ± 468	76
		DBA/2 (dddddd)	36,143 ± 1,590	15,143 ± 1,590	58
		C3H (kkkkkk)	39,549 ± 915	34,840 ± 953	12
		129 (bbbbbb)	48,931 ± 1,815	45,904 ± 2,463	5.1
3	C57BL/6 (bbbbbb)	None	8,422 ± 655	7,229 ± 577	14
		B6D2F1 (bbbbbb/dddddd)	13,981 ± 8,255	6,762 ± 803	52
		B6D2F1 (800R <i>in vitro</i>)	14,988 ± 6,353	5,605 ± 931	63
		DBA/2 (dddddd) (spleen)	14,278 ± 1,992	11,960 ± 670	16
		DBA/2 (thymus)	16,060 ± 210	16,530 ± 5,110	-3.0
		DBA/2 (marrow)	35,637 ± 2,282	30,298 ± 4,931	15

*Resistant and susceptible to the leukaemic and immunosuppressive effects of Friend virus⁷⁻⁹. 9×10^5 resistant and 1×10^5 susceptible cells per well. Thymocytes and marrow cells were used in experiment 3 as indicated. Cells were cultured in triplicate in 0.2 ml volume in RPMI 1640 medium containing 10% foetal calf serum, with or without 0.5 µg concanavalin A (Con A) per well, for 72 h in Falcon Microtest II plates. ³H-thymidine (0.5 µCi per well) was added 18 h before harvesting cultures with an automatic device^{8,9}. B6D2F1 spleen cells were irradiated with a ¹³⁷Cs source just before culture in experiment 2.

[†]300 Focus-forming units of NB-tropic Friend virus complex per well.

[‡]Represents c.p.m. ³H-thymidine incorporation, Con A minus no Con A, mean ± s.e.m.

§The lower case refers to the H-2 determinants K, IA, IB, IC, S and D.

cellular cooperation in antibody responses¹⁶⁻¹⁸ or in T cell-mediated lysis¹⁹ when the cell donors are tolerant to the antigens of the target cells. Even H-2-less cells can be lysed if concanavalin A is added to the reaction mixture, presumably to overcome a deficiency in 'recognition' of the target cell by the killer T cell²⁰. In appropriate conditions *in vivo*, that is, transfer of 'resistant' marrow cells into irradiated 'susceptible' infant mice previously infected with FV, we had not observed any H-2 restriction for immunosuppression in the FV model²¹. Therefore, we considered the possibility that a third cell type, other than the T suppressor cell or the mitogen-responsive cell, was responsible for the H-2 restriction. If that were true, one may be able to overcome the H-2D compatibility requirement by treatments *in vitro* and *in vivo* which would eliminate the interfering cell.

We were able to overcome the H-2D restriction simply by irradiating the cell suspension containing T suppressor cells (Table 2). Moreover, spleen cells from an unhealthy DBA/2 donor mouse with a tiny thymus were capable of suppressing C57BL/6 mitogen-responsive cells. This alerted

us to the possibility that adrenal steroids may remove the interfering cell, since steroids do cause severe hypoplasia of the thymus. Indeed, spleen cells from DBA/2 mice injected 2 d earlier with 2.5 mg cortisol were able to suppress mitogen-responsive cells of C57BL/6 mice in the presence of FV. Spleen cells from infant DBA/2 donors also suppressed H-2-incompatible C57BL/6 cells. To complete the analysis, we were able to 'reconstitute' the H-2 compatibility requirement by mixing adult untreated DBA/2 spleen cells containing the interfering cell with DBA/2 spleen cells devoid of such cells (Table 2).

We considered the possibility that an 'allogeneic effect' produced by interaction between interfering cells and H-2D-incompatible mitogen-responsive cells¹⁴ might have been responsible for these findings. However, semi-allogeneic F1 hybrid spleen cells from susceptible donors were able to suppress parental-strain spleen cells from resistant donors (Table 1). This result indicates that allogeneic effect could not be responsible for failure of H-2-incompatible cells to interact in this system.

The complex immune cellular interactions so far detected

Table 2 Effect of source of DBA/2 spleen cell suspension on suppression of C57BL/6 mitogen-responsive cells

Experiment	Nature of DBA/2 spleen cell suspension*	Change in Blastogenesis [‡]		Suppression %
		-FV [†]	+FV	
1	—	54,693 ± 9,889	54,513 ± 9,260	0.32
	Adult, 0 R	53,584 ± 9,526	54,165 ± 7,399	-1.0
	Adult, 1000 R	55,677 ± 1,292	32,192 ± 3,219	42
	1:1 0 R:1000 R	51,473 ± 2,450	55,541 ± 1,187	-7.9
	Infant	48,804 ± 3,926	33,629 ± 813	31
	1:1 Adult, 0 R:Infant	41,800 ± 1,757	53,982 ± 3,911	-29
2	—	58,358 ± 1,845	63,092 ± 3,354	-7.0
	Adult, Vehicle	53,830 ± 6,139	59,082 ± 1,789	-9.0
	Adult, Cortisol	34,874 ± 2,171	13,569 ± 2,001	61
	1:1 Vehicle:Cortisol	41,794 ± 5,849	40,720 ± 4,853	2.5

*Adult, 12 weeks of age; infant, 3 d of age. Spleen cells were exposed to 1000 R of ¹³⁷Cs γ rays *in vitro* just before initiation of culture. 10^5 DBA/2 cells were added to 9×10^5 C57BL/6 spleen cells from adult mice with con A and/or FV as described in legend for Table 1. Adult DBA/2 mice were injected with 2.5 mg cortisol (or saline vehicle) 2 d before the spleen cells were collected. DBA/2 mice are susceptible to FV and were the source of T suppressor cells.

[†],[‡]See Table 1.

in the Friend leukaemia virus model are depicted in Fig. 2 and are summarised as follows. Marrow-dependent cells of genetically resistant mice, but not of genetically susceptible mice, restrict the numbers and/or functions of T suppressor cells *in vivo*^{8,9} (Fig. 2a and b). T suppressor cells, even in the presence of syngeneic interfering cells, inhibit the proliferation of H-2D-identical mitogen-responsive cells in cultures infected with FV (Fig. 2c). Interfering cells, syngeneic with the T suppressor cells, prevent the suppression of H-2-incompatible mitogen-responsive cells (Fig. 2d). T suppressor cells, in the absence of syngeneic interfering cells, are quite capable of inhibiting the proliferation of H-2D-incompatible mitogen-responsive cells (Fig. 2e). Finally, mitogen-responsive cells of genetically resistant or susceptible mice proliferate well when exposed to FV, if no T suppressor cells are present in the mixture^{8,9}.

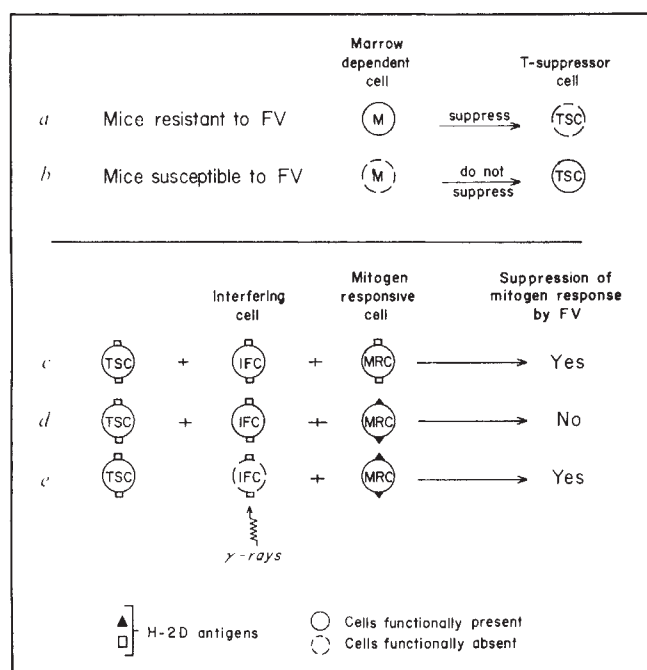


Fig. 2 Immune cellular interactions in the Friend leukaemia virus model. a, b: *in vivo*. c, d and e: *in vitro* cultures containing con A and FV.

One does not have to invoke 'altered self' or 'dual recognition' hypothesis to explain the H-2 compatibility requirements, at least in this instance. It is conceivable that the use of 'tolerant' cell populations to overcome the H-2 compatibility requirements in other studies¹⁶⁻¹⁹ could be explained by the elimination of an interfering third cell. The mechanism of interference by the third cell type has yet to be determined, but the release of receptors for allo-antigens²² is a hypothesis worth testing.

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Sister chromatid exchanges induced by mutagenic carcinogens in normal and xeroderma pigmentosum cells

XERODERMA pigmentosum (XP) is a human disease characterised by sensitivity to sunlight¹, a high incidence of skin cancer¹ and a deficiency in DNA repair². Investigations of DNA damage and repair in XP cells exposed to chemical carcinogens³⁻⁸ had led to the classification of these agents in two groups: those in which damage can be repaired by apparently normal levels of excision repair (for example, monofunctional alkylating agents) and those in which it cannot (for example, 4-nitroquinoline-1-oxide) (Table 1). In XP cells, normal excision of MMS⁹ or MNNG¹⁰ damage is correlated with normal levels of chromosome aberrations, and reduced excision repair of 4NQO damage is correlated with high levels of chromosome aberrations⁹, indicating that unrepaired damage is correlated with high levels of aberrations. It has recently been found that although sister chromatid exchanges (SCEs) differ from aberrations in many ways, they are far more sensitive indicators of chromosomal damage¹¹. We therefore considered that a comparison of excision repairability with the yields of SCEs induced by chemicals might be informative in elucidating mechanisms of SCE formation. The results indicate that, contrary to the results with aberrations, the frequency of induced SCEs is higher in XP cells than in normal cells whether or not XP cells can perform normal amounts of excision repair of the chemically-induced damage. The data indicate that the lesions and/or subsequent events that give rise to chromosome aberrations are different from those leading to sister chromatid exchange and that SCE formation in XP cells

Table 1 Relative levels of excision repair (damage excision, unscheduled synthesis, or repair replication)* in xeroderma pigmentosum cells damaged by various chemicals or ultraviolet light

Mutagen	Level of excision repair in XP cells (group A) (% of normal)	Reference
Ultraviolet light	< 5%	2
4-nitroquinoline-1-oxide	< 5%	4,6,7
Mitomycin C	reduced	20
Dimethyl sulphate	100%	30
Methyl methane sulphonate	100%	3,8
Ethyl methane sulphonate	100%	8,30
N-methyl-N-nitro-nitrosoguanidine	100%	3,6,7
Ethyl nitrosourea	100%	30

*These measurements of repair replication and unscheduled synthesis were all made during the first hours after exposure and represent measurement of the more rapidly repaired damage.

is the most sensitive mammalian system yet described for detecting possible genetic effects of chemicals.

Human fibroblasts from a normal donor (GM637, Human Genetics Cell Bank, Institute for Medical Research, Camden, New Jersey) and from an excision-repair deficient xeroderma pigmentosum patient (XP12RO, Complementation Group A, donated by A. Veldhuisen, TNO Rijswijk, The Netherlands) were treated with various concentrations of the chosen chemicals.

So that we could see sister chromatid exchanges, the cells were then grown for two rounds of DNA replication in the presence of 5-bromodeoxyuridine (BUdR) to produce "harlequin chromosomes" (ref. 12) in which the sister chromatids stained differentially.

The control yields of SCEs in normal and XP12RO cells are similar to one another (Table 2) and fall within the range previously reported for many cell types¹³⁻¹⁶, with the exception of the very high spontaneous yields in cells from patients with Bloom's syndrome¹⁴. The control yields in the present experiments, in which the cells were exposed to 20 μ M BUdR, are somewhat higher than the yields found after exposure to 10 μ M BUdR (ref. 15), which is consistent with the observation that some of the SCEs are caused by the BUdR itself¹⁷. Once exposed to the

chemicals, however, XP12RO cells contain higher numbers of SCEs than normal cells. This occurs even at concentrations too low to have detectable effects on normal cells (Table 2). The difference between normal and XP cells is most striking when MNNG (Fig. 1) is used, but is clearly evident for all the chemicals (Table 2). Kato noted that MNNG did not increase SCEs in normal Chinese hamster cells³¹.

Since XP cells cannot perform excision-repair of damage induced by 4NQO, the elevated levels of SCEs, as well as the elevated levels of chromosome aberrations^{9,10}, and increased cell killing⁸ observed after 4NQO treatment can be correlated with unrepaired damage. Such correlations also exist for ultraviolet damaged XP cells^{18,19}.

The same correlation cannot be made with damage induced by MMS, EMS, ENU, DMS, and MNNG, however, because XP and normal cells seem to undergo similar amounts of excision repair after exposure to these agents (Table 1). Nor can the correlation be made with damage induced by MMC because XP cells, which have been reported to be defective in unscheduled synthesis²⁰, respond as repair proficient cells for the induction of chromosome aberrations²⁰.

If XP12 cells are defective only in excision repair, then

Table 2 Chemically-induced sister chromatid exchanges in xeroderma pigmentosum and normal cells

Treatment	Normal cells (GM637) No. of SCEs/No. of chromosomes	SCEs per chromosome	Xeroderma cells (XP12RO) No. of SCEs/No. of chromosomes	SCEs per chromosome
Control	1369/4397	0.31	1785/4867	0.37
Ultraviolet-like				
4NQO				
4 $\times 10^{-10}$ M	744/2191	0.34	1764/2584	0.68
4 $\times 10^{-9}$ M	770/2156	0.36	2025/2271	0.89
X-ray-like				
MMS				
10 ⁻⁷ M	661/2141	0.31	1182/2106	0.56
10 ⁻⁶ M	710/2150	0.33	1256/2280	0.55
5 $\times 10^{-6}$ M	860/1981	0.43	1696/2161	0.78
EMS				
10 ⁻⁶ M	769/2299	0.33	1388/2459	0.56
10 ⁻⁵ M	797/2226	0.36	2399/2453	0.98
MNNG				
10 ⁻⁹ M	537/1726	0.31	1060/2313	0.46
10 ⁻⁸ M	875/2207	0.40	1494/2450	0.61
10 ⁻⁷ M	842/2057	0.41	4076/2519	1.62
10 ⁻⁶ M	1381/2136	0.65	Too numerous to be scored	
ENU				
10 ⁻⁶ M	773/2061	0.38	829/1734	0.48
10 ⁻⁴ M	1042/1932	0.54	2222/2231	1.00
DMS				
10 ⁻⁶ M	771/1917	0.40	978/2016	0.49
10 ⁻⁵ M	809/1824	0.44	1457/1977	0.74
5 $\times 10^{-5}$ M	1650/2367	0.70	2872/2153	1.33
10 ⁻⁴ M	2823/2286	1.23	4111/2222	1.85
MMC				
10 ⁻¹⁰ M	612/1621	0.38	994/2288	0.43
10 ⁻⁹ M	754/1793	0.42	1199/1965	0.61
5 $\times 10^{-9}$ M	1009/2074	0.49	2818/2320	1.21
10 ⁻⁸ M	1483/2232	0.66	4379/2357	1.86

The cell lines were both transformed by SV40 and were chosen because of their high rate of cell division; previous studies^{19,28} have demonstrated that transformation does not alter the DNA repair characteristics of human fibroblasts, although it does slightly increase the spontaneous frequency of sister chromatid exchanges in various cell types (normal, galactosaemic, Lesch-Nyhan and XP)¹⁵. Cells were grown in 75-cm² plastic flasks containing Eagle's minimum essential medium (MEM) with Earle's salts and 15% foetal calf serum. Twenty-four hours after the cells were recultured, BUdR (final concentration 20 μ M) and the carcinogens were added to the flasks. The cells were then grown for two rounds of DNA replication (48 h) so that both strands of one chromatid and one strand of its sister would contain BUdR. Once BUdR had been added, the cells were kept in total darkness except during handling procedures, which were carried out in subdued light from incandescent bulbs to minimise any effects caused by the photolysis of BUdR-containing DNA. The yields of SCEs under these conditions were no higher than those obtained under total darkness. The carcinogens used included ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), *N*-methyl-*N*-nitro-nitrosoguanidine (MNNG), ethyl nitrosourea (ENU), dimethyl sulphate (DMS), 4-nitro-quinoline-1-oxide (4NQO), and mitomycin C (MMC). These were dissolved in medium (EMS, MMS), in phosphate-buffered saline at pH 7.2 (MNNG, DMS) or at pH 6.0 (ENU), in ethanol (4NQO), or in water (MMC) and then diluted with MEM to obtain the final concentrations that were to be added to cells. Colcemid (final concentration 2 $\times 10^{-6}$ M) was added to each flask for the final 6 h of exposure of BUdR. The mitotic cells were then shaken off the flasks, centrifuged and treated with a hypotonic solution (0.075 M KCl) for 6 min to spread the chromosomes. The cells were centrifuged, and the pellet was fixed and washed twice in 3:1 methanol/acetic acid. The fixed cells were concentrated by centrifugation and dropped on to dry microslides. The slides were stained in 5 μ g ml⁻¹ of Hoechst 33258 in phosphate-buffered saline at pH 6.8 (ref. 29) and then washed in distilled water, thoroughly dried, and mounted with a drop of Sorensen's buffer (pH 6.8) and a coverslip. They were then exposed to light one and a half inches from a high pressure mercury lamp (625 J m⁻² s⁻¹) for 45 s to 5 min to allow the photochemical reaction to take place. The coverslips were then removed and the slides washed in distilled water, drained and then stained in 4% Giemsa (Gurr's R66) in M/15 Sorensen's buffer at pH 6.8, dried, and mounted in DePeX.

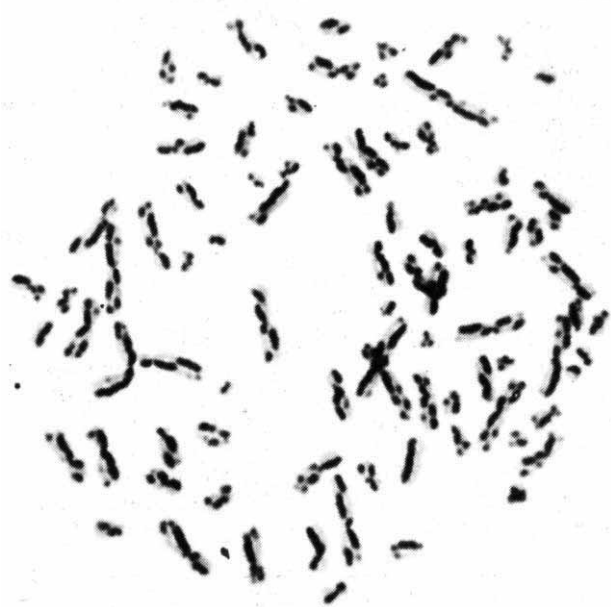


Fig. 1 A polyloid XP cell showing many SCEs after exposure to MNNG.

the increased yield of SCEs in such cells would be caused by unexcised damage in DNA. According to such a hypothesis, the results obtained with the alkylating agents would indicate that there is a minor fraction of the total damage remaining unexcised in each case. This fraction would be too small to be resolved by the usual measurements of repair replication or unscheduled synthesis, which have been made immediately after exposure to mutagens³⁻⁸, as well as too small to give rise to higher than normal amounts of chromosome aberrations^{9,10}, or cell killing⁶. An increase in SCEs, however, is induced since they are very sensitive indicators of unexcised damage in DNA.

These observations, and the apparent lack of correlation between SCE frequencies and either chromosome aberration frequencies or cell killing (in the case of the alkylating agents) raise questions about the biological significance of SCE formation. SCEs differ in many respects from chromosome aberrations: SCEs are induced at high frequencies by chemicals at concentrations that induce few aberrations¹¹; SCEs are not markedly increased by low doses of ionising radiations²¹, whereas aberration frequency is; SCEs saturate with increasing doses of ionising radiation²², and with tritiated thymidine²³; SCEs react differently to treatments with caffeine than do chromosome aberrations^{24,25}; SCEs are not correlated in any consistent fashion with the increased aberrations seen in the human diseases, Bloom's syndrome¹⁴ ataxia telangiectasia¹⁶ and Fanconi's anaemia²⁶, since SCE levels are very high in Bloom's, normal in ataxia telangiectasia, and reportedly low in Fanconi's anaemia.

SCEs may thus be the result of fundamentally different cellular events and lesions than are chromosome aberrations. Since the latter are associated with cell death, SCEs may be more representative of events compatible with cell survival, including mutagenesis. Although the majority of SCEs are probably genetically neutral because equal amounts of homologous chromatids are exchanged, some could conceivably be involved in deletion, insertion or frame shift mutagenesis because of a slight inequality of the exchanged material. Indeed, Magni and von Borstel²⁷ have found that mutation in yeast is correlated with meiotic exchange.

The sensitivity of XP cells we have observed indicates that whatever may be the genetic significance of SCEs, their measurement in XP cells is the most sensitive

indicator yet found for detecting chromosomal effects of potential mutagens and carcinogens in mammalian cells.

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Drugs which disrupt microtubules do not inhibit the initiation of lymphocyte activation

LYMPHOCYTES are activated to grow and divide when mitogens such as plant lectins bind to surface receptors. Several hypotheses have been advanced to explain how a signal which results from the interaction of an activating ligand with its receptors, may be transduced across the membrane to initiate cell activation¹. Recently Edelman and co-workers proposed a model in which a complex, composed of receptor, microfilaments and microtubules serves as a regulator for lymphocyte mitogenesis²⁻⁶. From experiments in which colchicine, when added to stimulated cultures at various time intervals, gradually lost its capacity to suppress subsequent DNA synthesis over 30-40 h, it was concluded that drugs which disrupt microtubules block lymphocyte activation at the point at which a lymphocyte becomes committed to undergoing growth and division^{7,8}. However, other similar experiments^{7,8} showed that colchicine was effective in suppressing DNA synthesis up to 48 h after the addition of mitogen. If indeed the microtubules are involved in the initiation of lymphocyte activation, then the disruption of these cytoplasmic structures should also affect cellular events which occur before DNA synthesis⁹.

Here we report that two microtubule active drugs, colchicine and vinblastine, failed to affect events which occur early in stimulated lymphocytes, namely the increased RNA synthesis, the synthesis of a specific protein, lymphotoxin, and the increased turnover of membrane phospholipids. These results strongly suggest that microtubules are

Table 1 The effect of colchicine or vinblastine on the concanavalin A-induced incorporation of ^3H -thymidine into DNA*

Concentration of drugs (M)	c.p.m. (10^{-3}) per 4×10^5 lymphocytes†	
	Colchicine	Vinblastine
Control	59.9±9.2	59.9±9.2
10^{-4}	15.7±1.0	0.6±0.9
10^{-5}	13.0±5.4	16.9±5.6
10^{-6}	18.3±0.9	17.7±5.1
10^{-7}	29.4±2.1	35.5±10.2
10^{-8}	39.8±7.9	52.3±3.5

* Incorporation into unstimulated lymphocytes: 0.6 ± 0.2 .† Data given are means of triplicates $\pm$ s.d. 4×10^5 lymphocytes were cultured in microplates in 0.2 ml DME supplemented with 10% FCS in a water-saturated air- CO_2 atmosphere to maintain a pH of 7.2–7.4. Concanavalin A ($2 \mu\text{g}$) and the microtubule active drugs were added at the beginning of the experiment. After 54 h of culture $1 \mu\text{Ci}$ ^3H -thymidine (specific activity 1 Ci mmol^{-1}) was added and the lymphocytes were incubated for further 16 h. DNA was collected with an automated sample harvester and the radioactivity retained on the glass filters was measured by liquid scintillation counting.

not involved in the process which is responsible for the commitment of lymphocytes.

The experiments were performed with lymphocytes from the mesenteric lymph nodes of 8–12-month-old rabbits which were grown under SPF conditions. For measuring DNA synthesis, cells were cultured at $2 \times 10^6 \text{ ml}^{-1}$ in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum (FCS) in microtitre plates. To determine RNA synthesis, lymphocytes at $1 \times 10^7 \text{ ml}^{-1}$ were cultured under similar conditions. The incorporation of phospholipid precursors into lecithin was measured in lymphocytes, cultured at $10^7 \text{ cells ml}^{-1}$ in DME supplemented with 2.5 mg ml^{-1} defatted albumin. Lymphotoxin was tested in supernatants from lymphocytes cultured at 10^7 ml^{-1} in DME+10% FCS for 24 h. In all experiments the microtubule active agents were added together with concanavalin A at the beginning of each experiment.

Table 1 shows that colchicine and vinblastine suppressed the incorporation of ^3H -thymidine into DNA of lymphocyte cultures stimulated with concanavalin A. Concentrations of 10^{-8} M and higher proved to be active. At concentrations of 10^{-5} the suppression of DNA synthesis ranged between 57 and 78% (mean 67%) with colchicine and between 63 and 82% (mean 72%) with vinblastine. In a concentration range up to 10^{-5} M both drugs were not toxic for lymphocytes after 4 and 20 h of culture, as assessed by the exclusion of trypan blue. At concentrations of 10^{-4} M , vinblastine started to be cytotoxic which was also found with colchicine at concentrations higher than 10^{-5} M .

The incorporation of ^3H -uridine into RNA becomes

measurable early in the course of activation. From measurements taken at 4 or 22 h, neither drug, at concentrations up to 10^{-5} M influenced the uptake of ^3H -uridine into RNA of stimulated lymphocytes (Table 2). A remarkable suppression of ^3H -uridine uptake was, however, always observed in unstimulated lymphocytes after 22 h. These data would favour the notion that colchicine as well as vinblastine acts on the permeation of ^3H -uridine into the cell, which is overcome in stimulated lymphocytes.

Lymphocytes which are activated by plant lectins are able to mimic the immune functions which are physiologically expressed after antigenic stimulation. In T lymphocytes, which are activated by concanavalin A, this includes the synthesis of biologically active proteins, the lymphokines. The secretion of one of the lymphokines, namely lymphotoxin, which causes impairment of cell proliferation or cell death was therefore measured in supernatants of lymphocytes stimulated with concanavalin A. After a culture period of 24 h lymphotoxin was present in stimulated cultures but not in controls (Table 3). The addition of colchicine or vinblastine at concentrations of

Table 3 The effect of colchicine and vinblastine on the secretion of lymphotoxin

Concanavalin A	Colchicine (10^{-5} M)	Vinblastine (10^{-5} M)	Inhibition of cell growth (%)
—	—	—	0
—	+	—	9.5 ± 0.5
—	—	+	10.3 ± 0.5
+	—	—	20.4 ± 1.0
+	+	—	25.3 ± 1.3
+	—	+	28.9 ± 1.5

Data are means of quintuplicates $\pm$ s.d. Lymphocytes were cultured at cell densities of $10^7 \text{ cells ml}^{-1}$ in DME supplemented with 10% FCS. Con A ($10 \mu\text{g ml}^{-1}$) and microtubule-disrupting drugs were added at the beginning of the experiments. After 24 h the lymphocytes were removed by centrifugation for 10 min at $350g$ and the cell-free supernatants (containing lymphotoxin) were collected. As target cells for the action of lymphotoxin a permanent cell line F1 (derived from the human placenta) was used. Lymphotoxin activity was determined by the inhibitory effect on the ^3H -thymidine incorporation into the target cells.

10^{-5} M only marginally affected the production of lymphotoxin, when measured by its capacity to suppress cell growth. Control incubations cultured in the presence of the microtubule active drugs alone also caused some reduction of target cell proliferation which, however, was much less apparent than those caused by the lymphotoxin. The measurement of growth reduction through biologically active substances in the presence of drugs which themselves inhibit proliferation may be difficult to interpret. Therefore the direct lytic effect of lymphokines (measured

Table 2 The effect of colchicine or vinblastine on the incorporation of ^3H -uridine into RNA

Hours of culture	Concentration of the drugs (M)	c.p.m. ($\times 10^{-3}$) per 2×10^6 lymphocytes*			
		Colchicine		Vinblastine	
		Control	Con A	Control	Con A
4	Control	1.67 ± 0.18	3.03 ± 0.51	1.67 ± 0.18	3.03 ± 0.51
	10^{-4}	1.40 ± 0.09	2.50 ± 0.12	0.83 ± 0.04	0.83 ± 0.50
	10^{-5}	1.75 ± 0.09	2.76 ± 0.23	1.43 ± 0.05	3.23 ± 0.15
	10^{-6}	1.58 ± 0.04	3.27 ± 0.11	1.67 ± 0.09	3.44 ± 0.16
22	Control	0.84 ± 0.10	4.20 ± 0.32	0.84 ± 0.10	4.20 ± 0.32
	10^{-4}	0.45 ± 0.13	3.14 ± 0.29	0.04 ± 0.01	0.40 ± 0.01
	10^{-5}	0.60 ± 0.02	4.17 ± 0.14	0.42 ± 0.03	4.00 ± 0.11
	10^{-6}	0.64 ± 0.10	4.36 ± 0.71	0.66 ± 0.02	4.40 ± 0.25
22†	Control	0.87 ± 0.15	5.20 ± 0.86	0.87 ± 0.15	5.20 ± 0.86
	10^{-5}	0.55 ± 0.04	5.00 ± 0.78	0.54 ± 0.16	5.20 ± 1.05

* Data are given as means of triplicates $\pm$ s.d.† Data given are means of three separate experiments $\pm$ s.d.

Lymphocytes (2×10^6) were incubated in 0.2 ml DME, supplemented with 10% FCS under conditions described in Table 1. After 2 or 20 h, $1 \mu\text{Ci}$ ^3H -uridine (50 mCi mmol^{-1}) was added and the cells were cultured for additional 2 h. The cells were then collected as described in Table 1.

by the release of ^{51}Cr) was analysed with lymphotoxin preparations, after separation by chromatography on Sephadex G-150. The lytic effect of partially purified lymphotoxin from lectin-stimulated lymphocytes, which was eluted with 70,000–90,000 molecular weight substances, cultured with or without 10^{-5} M colchicine or vinblastine, was identical ($18 \pm 5\%$ after 4 h).

Very early in lectin-activated lymphocytes, the turnover of phospholipid fatty acids increased (10–12). Concentrations of 10^{-4} M of colchicine or of 10^{-5} M of vinblastine were unable to suppress the incorporation of ^{14}C -oleate into lecithin of con A-stimulated lymphocytes when measured after 4 h of incubation (Table 4). In four separate experiments, where colchicine or vinblastine were present at concentrations of 10^{-5} M, the incorporation of ^{14}C -oleate into lecithin of con A-stimulated lymphocytes reached 99 ± 11 (colchicine) or 98 ± 7 (vinblastine) percent of controls (data given with standard deviation). The turnover of the phospholipid fatty acids may also be followed by labelling the long chain fatty acids biosynthetically with ^{14}C -acetate¹⁰. As shown in Table 4, colchicine at a concentration of 10^{-4} M, or vinblastine at a concentration of 10^{-5} M caused no decrease of the ^{14}C -acetate uptake into lecithin of con A-stimulated lymphocytes. However, at concentrations higher than 10^{-5} M, vinblastine markedly decreased the incorporation of ^{14}C -acetate into lecithin of resting lymphocytes. These findings suggest that vinblastine in high concentrations primarily interferes with the permeation of acetate into lymphocytes.

Table 4 The effect of colchicine or vinblastine on the incorporation of ^{14}C -oleate or ^{14}C -acetate into lecithin*

Concentration of the drugs (M)	^{14}C -oleate			
	mmol lecithin per 10^7 lymphocytes after 4 h culture†			
	Control	Concanavalin A	Control	Concanavalin A
Control	0.61	1.27	0.61	1.27
10^{-3}	0.61	1.11	—	—
10^{-4}	0.81	1.29	0.37	0.43
10^{-5}	0.78	1.41	0.84	1.31
10^{-6}	0.79	1.36	0.71	1.39

Concentration of the drugs (M)	^{14}C -acetate			
	c.p.m. (10^{-2}) in lecithin per 10^7 lymphocytes after 4 h of culture†			
	Control	Concanavalin A	Control	Concanavalin A
Control	13.9	25.0	13.9	25.0
10^{-3}	9.3	16.9	—	—
10^{-4}	13.8	26.0	6.2	6.3
10^{-5}	12.2	29.3	6.9	24.5
10^{-6}	13.8	25.4	10.4	23.7
10^{-7}	—	—	13.7	23.9

* This fraction also contains smaller contaminants of phosphatidylserine and phosphatidyl inositol.

† Data are means of duplicates.

Lymphocytes (10^7) were incubated at 37°C in 1 ml HEPES-buffered DME supplemented with 2.5 mg defatted albumin.

^{14}C -oleate (10 nmol) or 25 nmol ^{14}C -acetate were added at the beginning of the experiment. After incubation the lipids were extracted, separated by thin-layer chromatography as described previously, and incorporation into lecithin was measured by liquid scintillation counting¹⁰.

When lymphocytes are activated by mitogens as the lectin concanavalin A, a sequence of metabolic events is initiated which finally leads to cell division. Our results confirm earlier reports that colchicine or vinblastine at concentrations which disrupt the microtubular system suppress incorporation of ^3H -thymidine into lectin-stimulated lymphocytes. Addition of colchicine 30 h after the mitogen proved to suppress subsequent DNA synthesis to the same degree as when concanavalin A and colchicine were added together at the beginning of an experiment (data not presented). Cellular changes, which occur before the initiation

of DNA synthesis were not affected in stimulated lymphocytes in the presence of colchicine or vinblastine. The incorporation of ^3H -uridine into stimulated lymphocytes was not suppressed at concentrations of 10^{-5} M. Similar results have been reported recently by Betel *et al.*⁸. Furthermore, the secretion of lymphotoxin which is induced in activated lymphocytes, was not decreased in a similar concentration range. In addition, cellular changes which are induced very rapidly after the binding of a mitogen to the surface receptors, that is, the increased turnover of the membrane phospholipids, were similarly not inhibited by concentrations of colchicine as high as 10^{-4} M or 10^{-5} M vinblastine. Thus our data are not compatible with the concept that microtubule-disruptive agents prevent the initiation of lymphocyte activation. It therefore seems unlikely that the putative association of receptor molecules with microtubules plays a large part in the signal transmission in concanavalin A-activated lymphocytes.

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Habitat values and endemism in the vanishing rain forests of Sri Lanka

TROPICAL forests are among the most seriously threatened terrestrial environments, and while biologists and conservationists argue for preservation, the plunder continues virtually unabated¹. In Sri Lanka (Ceylon), for example, the southern rain forests and montane forests contain a rich assemblage of endemic animals, but despite the protestations of the local conservationists, deforestation is rapidly destroying these habitats. One reason for government inaction in Sri Lanka, as elsewhere, may be the inability of conservationists to make an objective case for the value or uniqueness of these forests. In this paper, we propose some new approaches to this old problem.

Habitat value judgments must be quantitatively expressed if they are to seem credible in this technological age. The dilemma is that all estimates of quality or value are inextricably rooted in cultural, ethical and aesthetic assumptions, which are themselves value judgments. So-called objectivity in such matters is obviously impossible even though agreement is often achieved, at least within a culture. The logical and epistemological problems notwithstanding, we suggest in this paper some indices of habitat value that have the virtues of simplicity, flexibility and (to us, at least) intuitive appeal. The simplest and most general form of the index is

$$V_i = (Q_i/A_i) 1,000 \quad (1)$$

when A_i is area of the i th habitat and Q_i is the number of species in

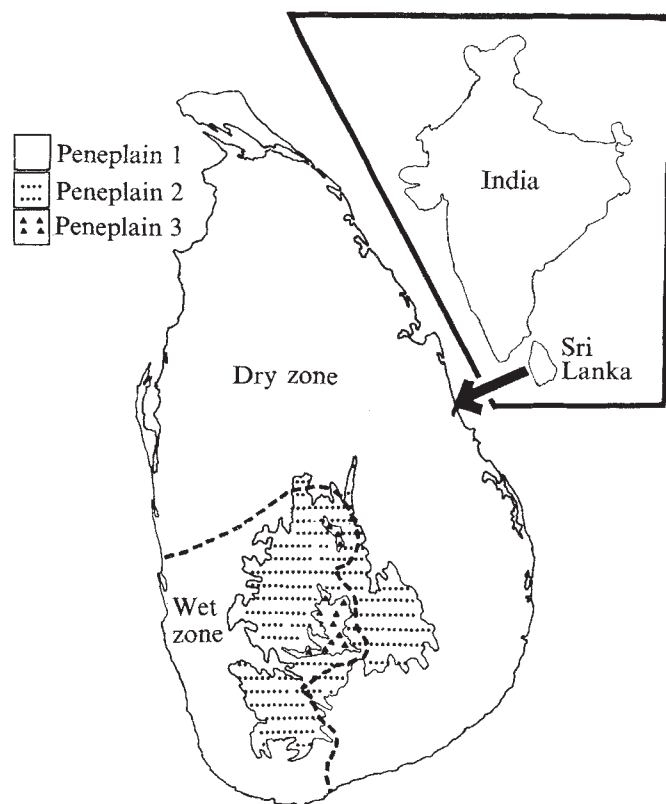


Fig. 1 The island of Sri Lanka showing the three peneplains and the dry and wet zones. The dashed line encloses the wet zone. The dry zone of peneplain 1 corresponds to region A; the wet zone of peneplain 1 to region B; peneplain 2 corresponds to region C; peneplain 3 corresponds to region D.

the i th habitat which have some quality deemed desirable. Depending on the location and purpose, "desirable" species could be those that are endemic, rare, large, or ancestors of cultivars, to list a few examples.

Under some circumstances, it would be appropriate to replace Q_i with a term that is the sum of desirability rankings. For example, endangered species occurring nowhere else might receive a rank of 10; an endangered species that occurs in the other habitats or regions might receive a rank of 5; rare species, but not officially endangered species, might receive a rank of 1. A similar but less arbitrary procedure is to employ the index

$$V'_i = \left(\sum \frac{1}{A_j} \right) 1,000 \quad (2)$$

when A_j is the area of the total geographic range of the j th species occurring in habitat i . This index has the advantage of being free of official judgments about the status of species and incorporating the commonest criterion for concern, namely, geographically restricted distribution. This approach would not be as appropriate as the ranking method for organisms, like some birds, which can simultaneously be both rare and widespread.

An important feature of index (1) (but not index (2)) is that the value of a habitat increases as the area it occupies decreases, even when there is no change in the number of species. Therefore, this index will be especially useful in comparing large areas with smaller, putatively more valuable ones. Conversely, it will not be very helpful when considering a large chunk of undifferentiated wilderness. Conservation arguments for the latter will often need to stress other considerations, including the space requirement of large, migratory species, the advisability of preserving large amounts of genetic variation in some species², and the needs of present and future generations for wilderness.

Figure 1 is a map of Sri Lanka showing the major

biogeographical regions as defined by altitude, rainfall, and flora^{3,8}. The island has been divided into three 'peneplains'. This term refers to well-marked plains of erosion. The lowest peneplain is by far the largest and surrounds the south-central hill country. The average elevation of this area is around 30 m, but hills within it reach 125 m. The second peneplain rises as a steep step from the first and averages around 400-500 m. The third peneplain is around 1,650 m.

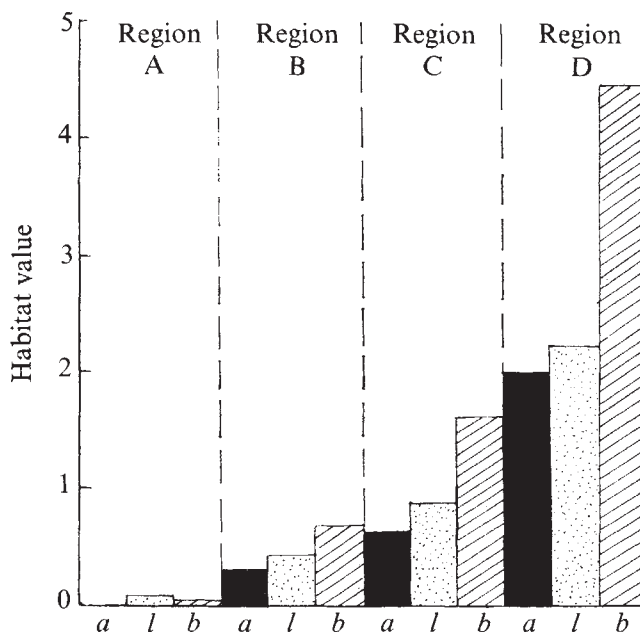
Precipitation is dependent on the trade winds and is seasonal. Two monsoons (rainy seasons) occur in Sri Lanka, the south-west (May to September) and the north-east (November to February). The rains of the south-west monsoon tend to be contained in the southern and south-western part of the island by the central mountain massif, while the north-east monsoon brings rains to both areas. As a consequence, two humidity 'zones' can be distinguished: the dry zone, that part of the island that receives only the north-east monsoon rains and an annual rainfall of under 190 cm; the wet zone which receives both monsoons and has an annual rainfall of over 190 cm. The second and third peneplains lie within the wet zone while the first peneplain is represented in both zones.

Based on the above geological and climatic zones, most authors have subdivided Sri Lanka into four biogeographic regions. We refer to these as follows: region A, the dry northern and eastern areas of the first peneplain or the dry zone; region B, the humid south-western part of the first peneplain; region C, the second peneplain; and region D, the third peneplain. Botanists have recognised floristic groupings that tend to reinforce this classification.

Recently, one of the authors (F.R.S.) spent 18 months on the island studying the distribution of reptiles and amphibians. The data indicate that the rain forest areas together with the montane regions in the south contain more endemic forms than any other part of the island. A similar pattern was observed in the avifauna. cursory investigation of the insects and fishes seems to demonstrate a parallel distribution pattern.

Table 1 gives a list of endemic amphibians, lizards, and birds of Sri Lanka tabulated according to their occupation of the above defined regions. The methods on which the taxonomic decisions are based will be described elsewhere. We have chosen birds, lizards and amphibians as examples because information on their distributions is superior to that of other groups; for

Fig. 2 Habitat values (index (1)) of the four biogeographic regions in Sri Lanka: a indicates amphibia; l indicates lizards; b indicates birds.



example, Carl Gans, in the course of his work on the uropeltid snakes, is finding the published distributions for snakes to be very inaccurate. The available data⁹⁻¹³ suggest that the patterns observed for the lizards, amphibians and birds ought to be reflected in the freshwater fishes and mammals.

In Table 2, we apply the habitat value indices to the Sri Lanka data; we consider only the species that occur nowhere else but on

Sri Lanka. These endemics have considerable biogeographic and ecological significance because most of them are phylogenetic relicts whose closest relatives occur in South Africa, Madagascar, the Comoro Islands, Malaysia, and Sumatra. In employing V_i we should, strictly speaking, base our calculations on all the species on Sri Lanka, whether endemic or not. We chose not to do this for two reasons: first, the distributions of many forms is poorly

Table 1 Endemic amphibians lizards and birds of Sri Lanka

	Region			
	A	B	C	D
Amphibians				
<i>Bufo kelartii</i>			X	
<i>B. atukoralei</i>		X		
<i>Rana greenii</i>			X	X
<i>R. corrugata</i>		X	X	
<i>R. gracilis</i>		X		
<i>Nannophrys guntheri</i>		X		
<i>N. ceylonensis</i>			X	
<i>N. marmorata</i>				X
<i>Rhacophorus cruciger</i>		X	X	
<i>R. eques</i>				X
<i>R. microtympaum</i>				X
<i>R. nasutus</i>			X	
<i>Philautus schmardanus</i>				X
<i>Ramenella obscura</i>			X	X
<i>R. palmata</i>				X
<i>Microhyla zeylanica</i>				X
Lizards				
<i>Calotes calotes</i>	A	B	C	D
<i>C. liolepis</i>		X		
<i>C. liocephalus</i>			X	
<i>C. ceylonensis</i>	X			X
<i>Ceratophora aspera</i>			X	
<i>C. stoddartii</i>				X
<i>C. tennentii</i>				X
<i>Cophotis zeylanica</i>				X
<i>Lireocephalus scutatus</i>			X	
<i>Otocryptis weigmanni</i>		X		
<i>Cnemaspis podihuna</i>	X			
<i>C. jerdonii</i>			X	
<i>Gymnodactylus frenatus</i>			X	
<i>Geckoella trierdrus</i>				X
<i>G. yakhuna</i>			X	
<i>Hemidactylus depressus</i>	X			
<i>Mabuya macularia</i>		X		
<i>Dasia haliana</i>	X			
<i>Sphenomorphus striatopunctatus</i>				X
<i>S. taprobens</i>		X		
<i>S. deigani</i>			X	
<i>S. dorsicatenatus</i>			X	
<i>S. ffallax</i>		X		
<i>Chalcidoseps thwaitesi</i>				X
<i>Nessia butoni</i>			X	
<i>N. didactyla</i>		X		
<i>Evesia monodactyla</i>				X
<i>Bipedos sarasinorum</i>		X		
<i>B. smithi</i>				X
<i>Anguinicephalus layardii</i>			X	
<i>A. kickanale</i>	X			
Birds				
<i>Cissa ornata</i> (Ceylon Blue magpie)				X
<i>Turdoides rufescens</i> (Ceylon Rufous Babbler)		X	X	X
<i>Turdoides cinereifrons</i> (Ashy Headed Babbler)			X	X
<i>Pycnonotus melanicterus</i> (Black Capped Bul-Bul)			X	X
<i>Kelaartia penicillata</i> (Yellow Eared Bul-Bul)	X	X		X
<i>Oreocincla spiloptera</i> (Spotted Winged Thrush)		X	X	X
<i>Arrenga blighi</i> (Ceylon Whistling Thrush)			X	X
<i>Muscicapa sordida</i> (Dusky Blue Flycatcher)			X	X
<i>Bradypterus palliseri</i> (Ceylon Warbler)			X	X
<i>Eulabes ptilogenys</i> (Ceylon Grackle)			X	X
<i>Zosterops ceylonensis</i> (Ceylon Hill White Eye)				X
<i>Sturnia senex</i> (White Headed Starling)			X	X
<i>Dicaeum vincens</i> (Legges Flower Pecker)		X	X	X
<i>Cyanops flavifrons</i> (Yellow Fronted Barbet)			X	X
<i>Tockus gingalensis</i> (Ceylon Grey Hornbill)		X	X	X
<i>Phaenicophaeus pyrrhocephalus</i> (Red Faced Malkoha)	X	X	X	
<i>Centropus chlororhynchus</i> (Green Billed Coucal)		X	X	
<i>Psittacula calthorpeae</i> (Layards Parakeet)		X	X	X
<i>Loriculus beryllinus</i> (Ceylon Lorikeet)		X	X	
<i>Columba torringtoni</i> (Ceylon Wood Pigeon)			X	X
<i>Gallus lafayetii</i> (Ceylon Jungle Fowl)	X	X	X	X
<i>Galliperdix bicalcarata</i> (Ceylon Spur Fowl)		X	X	X

Table 2 Habitat value data and indices for the amphibians, lizards and birds of Sri Lanka

Region	Area (km ²)	Endemic amphibians			Endemic lizards			Endemic birds			Totals		
		No.	V_i	V_i'	No.	V_i	V_i'	No.	V_i	V_i'	No.	V_i	V_i'
A	66,593	0	0	0	5	0.08	0.08	3	0.04	0.03	8	0.12	0.11
B	16,058	5	0.31	0.26	7	0.44	0.44	11	0.69	0.30	22	1.43	1.00
C	11,266	7	0.62	0.57	10	0.89	0.89	18	1.60	0.75	35	3.11	2.21
D	4,048	8	1.97	1.61	9	2.22	2.22	18	4.45	1.65	35	8.65	5.48

known; and second, the results would not be changed significantly because of the very small value of $1/A$ for non-endemic species relative to endemic species.

For the amphibians and lizards, the two indices give nearly identical results. For the birds, however, the V_i' values are relatively small because of the wider distributions of the birds compared to the amphibians and reptiles. In Fig. 2, we illustrate the V_i values for the regions described above. In words, the meaning of the figure is that the highlands are approximately 50 times more valuable than the lowlands because the area available is so small and the number of desirable (endemic) species in the highlands is so high. The pattern of distribution we have illustrated for the endemic species also holds true at the generic level. Of the nine endemic genera of lizards and amphibians, eight were confined to the moist or montane zones, while only one genus occurs in all zones. Current data^{9,14,15} on the freshwater fishes and mammals indicate a similar trend at the generic level in these groups.

The data in Table 2 reinforce the opinion held by many biogeographers that the southern forests and highlands of Sri Lanka are a unique biological treasure trove of evolutionary relicts¹⁶. The habitat values tell the story. Darlington¹⁷ suggested that the number of relict forms on the island may be no more than occur in the southern part of India, but our recent studies on the reptilian and amphibian fauna and published data^{9,10} seem to indicate that Sri Lanka does indeed have a much higher number of relict forms than continental south India. Birds may be an exception, however, because of their greater mobility and corresponding higher rates of gene flow and colonisation.

The affinities of the endemic genera of reptiles and amphibians (some have congeners in Malaysia and Sumatra, others have their closest relatives in Africa and Madagascar) are noteworthy in light of recent attempts to interpret biogeographic patterns in terms of continental drift¹⁸. While the majority of total faunal representation is markedly Indian in origin, the percentage representation of the Indian element is much lower in the montane areas than in the lowland dry zone. Cruz¹⁵, in our opinion, rightly argues that the montane zones represent the most conservative elements of the Sri Lankan fauna, those that have been least influenced by recent invasions from the Indian mainland.

The habitat values of the wet, montane regions, while relatively high, are quite conservative because the percentage of land that remains forested in the wet, montane regions (B, C, and D) is only 9% compared to over 30% of the total area of region A. It should be emphasised that the paucity of endemics in the lowlands is in large part a consequence of periodic land bridge connections to South India in the Pleistocene and the episodic exchanges of lowland biotas.

Sri Lanka, like most tropical developing countries is today faced with rapid population growth and the problems associated with the cultivation of agriculturally marginal lands and the intensified exploitation of natural resources. The forests are now being cleared for the production of plywood and pulp. Extinction of many animals will inevitably occur because the ecological successional process that follows deforestation leads to the development of habitats unsuitable to most of the endemic forms (to be discussed more fully elsewhere). Even nationally accredited areas of unique habitat have recently been brought under the axe and plough¹⁵. Biologists, geographers and conservationists

should accomplish what research they can there before it is too late.

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Immunosuppression by mouse sialylated α -foetoprotein

AFP is the dominant serum protein in early embryonic life. It is considered also to be a tumour-associated antigen because it appears in the sera of patients and animals with primary liver cancer¹. The function of AFP in the foetus remains unknown. Tomasi *et al.*² have demonstrated that mouse AFP has an immunosuppressive effect on antibody synthesis both *in vivo* and *in vitro*. Similarly, human AFP has been reported to have immunosuppressive properties³. One of us (E.F.Z.) has shown that mouse AFP is heterogeneous, consisting of five components (Fp1–Fp5)⁴, which differ in the amount of sialic acid attached to the molecule: Fp1 contains no detectable sialyl residues while Fp5 has four per molecule⁵. We have now demonstrated that the presence of sialic acid residues in the AFP molecule is essential for the immunosuppressive activities of this glycoprotein.

The individual components of AFP were isolated as described before⁶. Briefly, mouse amniotic fluid or foetal plasma from days 15.5–16.5 of gestation were used as sources of α -foetoprotein. Components Fp1 and Fp5, respectively, were purified by electrophoresis on long preparative polyacrylamide gels at low amperage for 65 h in the cold room. Wedges were removed, the locations of AFP components were determined by fluorescence with ultra-violet-activated acidified anilino-naphthalene-sulphonic acid

reagent, and the wedges were aligned with the untreated gels. Portions of the untreated gels corresponding to Fp1 and Fp5 were cut out and eluted with 0.05 M Tris-0.1 M NaCl, pH 7.0. Fp1 and Fp5 components were used in tests for immunosuppressive properties because they have no residues and four residues, respectively, per molecule. Because of the possibility that Fp1, derived from amniotic fluid, might lack internal sugars, Fp5 was treated with neuraminidase. The glycoprotein lacking the terminal sialic acid, designated Fp1ⁿ, should now have its penultimate sugars on the oligosaccharide. Fp1ⁿ was separated from the neuraminidase-resistant Fp3 and as indicated later it migrated to the same position as that of Fp1.

The immunosuppressive activity of the components was determined in mouse splenic lymphocyte cultures in which the primary immune response to sheep erythrocytes was measured. Female BDF₁ mice, 10–12 weeks old (Jackson Laboratory) served as the source of splenic lymphocytes. Splenic cultures were prepared as described by Mishell and Dutton⁷. Spleen lymphocytes were cultured with sheep erythrocytes for 5 d in the presence or absence of AFP components. The number of antibody-forming cells to sheep erythrocytes on day 5 was determined by Jerne's plaquing technique⁸. As established by the Trypan blue dye exclusion test, AFP components were not cytotoxic for splenic lymphocytes. Figure 1 illustrates AFP components as identified before and after electrophoretic purification. Electrophoretic analysis of Fp5 after treatment with neuraminidase indicates that it now is very closely related or identical to the Fp1 component. Furthermore, no apparent contamination of each purified AFP component was observed.

Figure 2 shows precipitin analysis of the AFP components by double diffusion technique in agar gel. Goat antiserum prepared against components Fp1–Fp3 was tested against homologous and heterologous AFP components. A total identity band between the various AFP components is evident indicating that all AFP components possess a common structure. Mouse serum from adult animals did not show a precipitin band formation with anti-AFP antiserum indicating that our AFP preparations were not contaminated with other serum proteins.

The immunosuppressive effects of AFP components are shown in Table 1. Mouse amniotic fluid (MAF) and component Fp5 were significantly immunosuppressive: MAF (100 µg), $P < 0.05$; Fp5 (1 µg), $P < 0.0005$. Components Fp1 and Fp1ⁿ, however, exerted no effect on antibody formation to sheep erythrocytes. In addition, the acrylamide gel eluate, from which these AFP components were isolated, showed no immunosuppressive properties. Normal serum obtained from adult mice (NMS) was quite immunosuppressive although it contained no AFP (Fig. 2). Apparently

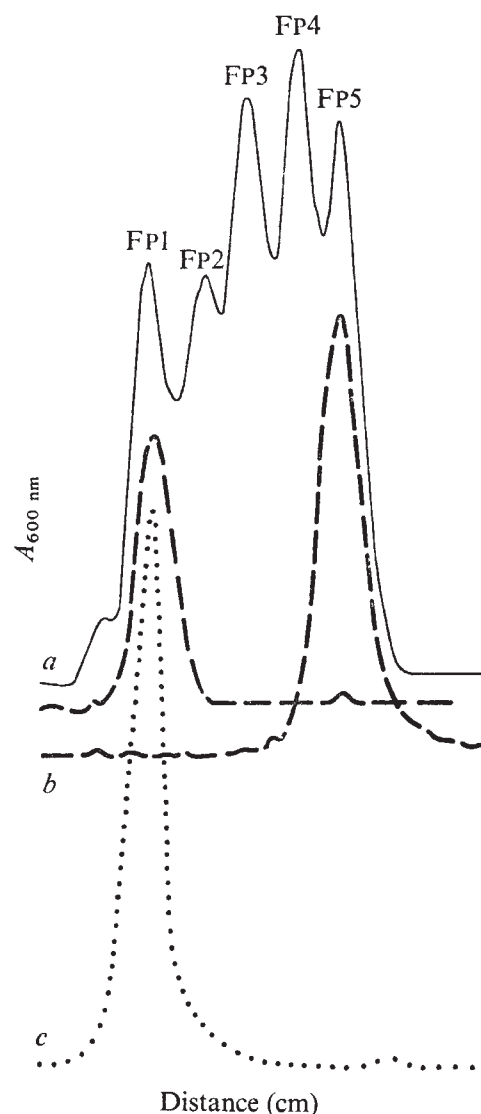


Fig. 1 Mouse α -foetoprotein from various preparations was separated by polyacrylamide gel electrophoresis as described before⁹. Electrophoresis was on 0.6×15 -cm gels for 2 h at 3.5 mA per gel and then 2 h at 2.6 mA per gel. Gels were stained with aniline-blue-black, diffusion destained, and absorbance was measured at 600 nm on a recording spectrophotometer with a gel scanner. Areas of gels containing α -foetoproteins are presented. *a*, Mouse foetal plasma (40 µg) from day 16.5 of gestation was the source of α -foetoprotein. Full scale absorbance was 2 units (—). *b*, Fp1 and Fp5 were each purified from day 15.5 amniotic fluid on preparative polyacrylamide gels (1.8×44 cm) with electrophoresis for 65 h at 9.1 mA per gel in the cold room. Fp1 and Fp5 were each (25 µg) electrophoresed to indicate purity. Full scale absorbance was 3 units (— — —). *c*, Fp1ⁿ was derived by treatment with *V. cholerae* neuraminidase from Fp5 purified from newborn plasma. Fp1ⁿ was then separated from Fp3ⁿ on a preparative gel, as described above, and Fp1ⁿ electrophoresed on an analytical gel to indicate purity. Full scale absorbance was 3 units (.....).

additional immunosuppressive factors are present in normal animal sera as demonstrated before⁹.

The apparent requirement for sialyl residues on the AFP molecule to achieve immunosuppressive properties parallels observations made in other biological systems. Remold and David have demonstrated¹⁰ that macrophage inhibitory factor (MIF) which is produced by sensitised lymphocytes requires the presence of terminal sialyl residues for its inhibitory activity. It is conceivable that sialyl residues, which are quite often found on the surface of cellular membranes and serum proteins, may have an important role in the regulation of various biological properties. Because we used a primary lymphocyte culture for antibody

Table 1 Effect of AFP components on the *in vitro* primary immune response to sheep erythrocytes

Preparation	PFC per 10 ⁶ viable cells $\pm$ s.e.
Control	597 $\pm$ 135
Gel eluate	530 $\pm$ 132
MAF (200 µg)	271 $\pm$ 82
MAF (100 µg)	365 $\pm$ 368
MAF (10 µg)	567 $\pm$ 8
Fp5 (10 µg)	284 $\pm$ 19
Fp5 (1 µg)	248 $\pm$ 96
Fp5 (0.1 µg)	506 $\pm$ 86
Fp1 (10 µg)	648 $\pm$ 168
Fp1 (1 µg)	467 $\pm$ 137
Fp1 ⁿ (10 µg)	579 $\pm$ 228
Fp1 ⁿ (1 µg)	709 $\pm$ 130
Fp1 ⁿ (0.1 µg)	588 $\pm$ 134
NMS (1%)	521 $\pm$ 150
NMS (5%)	168 $\pm$ 38
NMS (10%)	130 $\pm$ 104
NMS (15%)	66 $\pm$ 11

The values were derived from at least two experiments, each of which contained triplicate determinations.

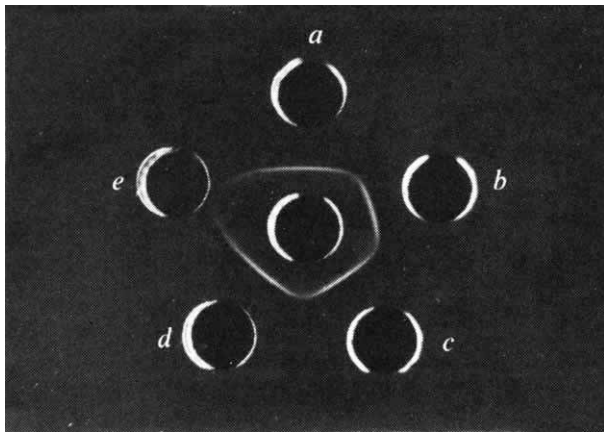


Fig. 2 Ouchterlony double immunodiffusion on an agar plate (Hyland) of α -foetoprotein preparations against anti-Fp1-3 (ref. 4). Source and purity of isolated α -foetoprotein preparations are indicated in Fig. 1. Outside wells contained: a, Fp5 (600 $\mu\text{g ml}^{-1}$); b, Fp1 (675 $\mu\text{g ml}^{-1}$); c, Fp1ⁿ (550 $\mu\text{g ml}^{-1}$); d, day 14.75 mouse amniotic fluid (2.2 mg ml^{-1}), and e, mouse adult serum (undiluted). The centre well contained the antiserum.

formation to sheep erythrocytes, it can be assumed that in our experimental conditions IgM immune response was suppressed. Dattwyler *et al.*¹¹ have reported that AFP binds specifically to T lymphocytes and therefore this type of lymphocyte (or perhaps a subpopulation of T lymphocytes) may carry on its surface receptors for sialyl residues. The mechanism of immunosuppression by AFP or other serum factors remains unknown. It has been reported that immunosuppressive properties of mouse AFP are affected by binding of oestrogen¹². It remains to be determined whether this binding is dependent on the presence of sialyl residues on AFP and whether the immunosuppression may involve more than one reactive substance. It is hoped that, as the structures in the lymphocyte membrane which can interact with sialyl residues are identified, the understanding of immunosuppressive processes will improve.

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Immunocytochemical studies of cells in the rat adenohypophysis containing both ACTH and FSH

STUDIES by Siperstein *et al.*^{1,2} first described the cytology of the adrenocorticotropin (ACTH) cell in the rat adenohypophysis. This cell is stellate and contains peripherally organised granules averaging 220 μm in diameter. Their cytological work was confirmed in later immunocytochemical studies in our laboratory³⁻⁵, in which ACTH was

localised in the secretory granules of cells of this type. In our recent immunocytochemical studies of follicle-stimulating hormone (FSH) cells, however, FSH was found in a cell which resembled the ACTH cell. We termed this cell the Type III gonadotroph^{6,7}. Here we describe studies designed to test the specificity of the reaction for FSH in this cell type, and the relationship between the Type III gonadotroph and the cell containing ACTH.

The immunocytochemical technique used was the unlabelled antibody peroxidase-antiperoxidase complex (PAP) technique of Sternberger *et al.*⁸, as modified in our laboratory for the ultrastructural localisation of ACTH^{3,4}. Pituitaries were fixed in picric acid formaldehyde and embedded in Araldite 6005. Ultrathin serial sections were mounted on nickel grids, etched in 10% H_2O_2 for 5-10 min, and then stained immunocytochemically.

The specificities of the immunocytochemical stains were determined by the absorption of each antiserum with ACTH or FSH for 2 d before its use in the stain. Table 1 lists the results of specificity tests of the anti-ACTH₁₇₋₃₉ sera and anti-rat FSH β sera. Only previous absorption with ACTH abolished staining in the ACTH cell with the anti-ACTH₁₇₋₃₉ serum; previous absorption with FSH had no effect. Similarly, FSH absorption abolished FSH staining in the Type III gonadotroph; however, absorption with ACTH had no effect.

Other specificity tests included the substitution of antiserum to luteinising hormone (LH) or thyroid-stimulating hormone (TSH) for the anti-FSH or anti-ACTH sera. Cells resembling the Type III gonadotroph were not abundant in either the LH cell population^{6,7} or the TSH cell population in normal male rats or cycling female rats. The omission of the goat anti-rabbit IgG from the staining sequence abolished staining completely.

After proof of specific staining was established, the next phase of the study was designed to test if the ACTH cell and the Type III cell were the same cell. For this purpose, the stains for ACTH₁₇₋₃₉ and FSH β were used on serial sections of oestrous female rats (1300 h on day of oestrus), which have an abundance of Type III gonadotrophs. Twenty serial cells were collected and analysed for their hormone content. Of the group of cells collected, nine stained for ACTH alone (Fig. 1). The other eleven cells stained for both FSH β and ACTH (Figs 1 and 2). Out of the cells analysed, we have yet to find a Type III gonadotroph which contains only FSH β .

The insets and arrows in Fig. 2 indicate that we have serially sectioned some of the granules. An analysis of the staining pattern of each of 26 granules in 5 areas of the cell depicted in Fig. 2 shows that 12 contain only ACTH, 8 contain both ACTH and FSH, and 6 are negative. No granules were found which stain for FSH only.

In summary, it is evident that one of the FSH cell types is a cell morphologically identical to the ACTH cell, and the serial sections collected thus far indicate that this cell contains ACTH as well. Our findings are difficult to explain. The staining for FSH β in a stellate cell can be explained from the standpoint of morphological differentiation. For example, in a particular physiological state, such as enhanced secretion, FSH gonadotrophs may become stellate following stimulation as an aid or adjunct to their activity. The finding that these cells contain ACTH as well is however, somewhat surprising since there is no known chemical homology between ACTH and FSH.

Sectioning 220-250- μm granules serially is difficult, and we were fortunate to obtain such a serial for the cell in Fig. 2. Our results show that when FSH staining is exhibited by a granule, it is found in a granule which also contains ACTH. If this staining simply reflected a subpopulation of antibodies to ACTH found in the anti-FSH serum, then one might expect that ACTH absorption would abolish it. The results in Table 1 show that ACTH absorp-

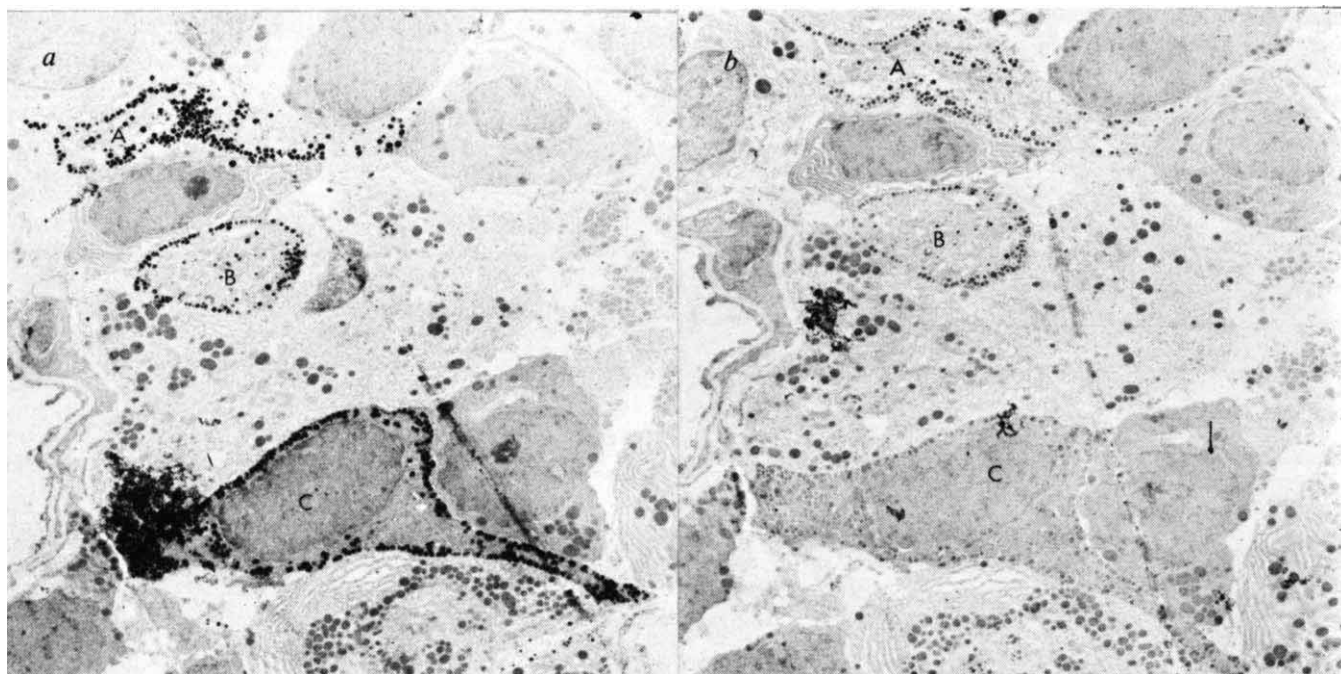


Fig. 1 *a*, Ultrathin section of rat pituitary stained immunocytochemically with 1:5000 dilution anti- ACTH_{17-39} serum (48 h, 4 °C) followed by 1:100 dilution goat anti-rabbit IgG, (3 min) followed by 1:100 dilution PAP solution (3 min) and the histochemical reaction for peroxidase 3, 4. *b*, Section serial to that in *a*, stained with 1:2000 dilution anti-rat FSH β followed by the same sequence of solutions as in *a*. Cell A stains intensely for ACTH and weakly for FSH β . Cells B and C stain intensely for ACTH alone. Cell A resembles a gonadotroph (Type II)⁷. Only a few granules in Cell B stain for FSH β ; cell C clearly does not react with anti-FSH β . Fixation: picric acid formaldehyde; embedded in Araldite 6005. Magnification: $\times 4500$.

tion had no significant effect on anti-FSH staining, however. Therefore, this suggests that the staining reflects the

presence of FSH or a molecule with some amino acid sequences identical to those in FSH.

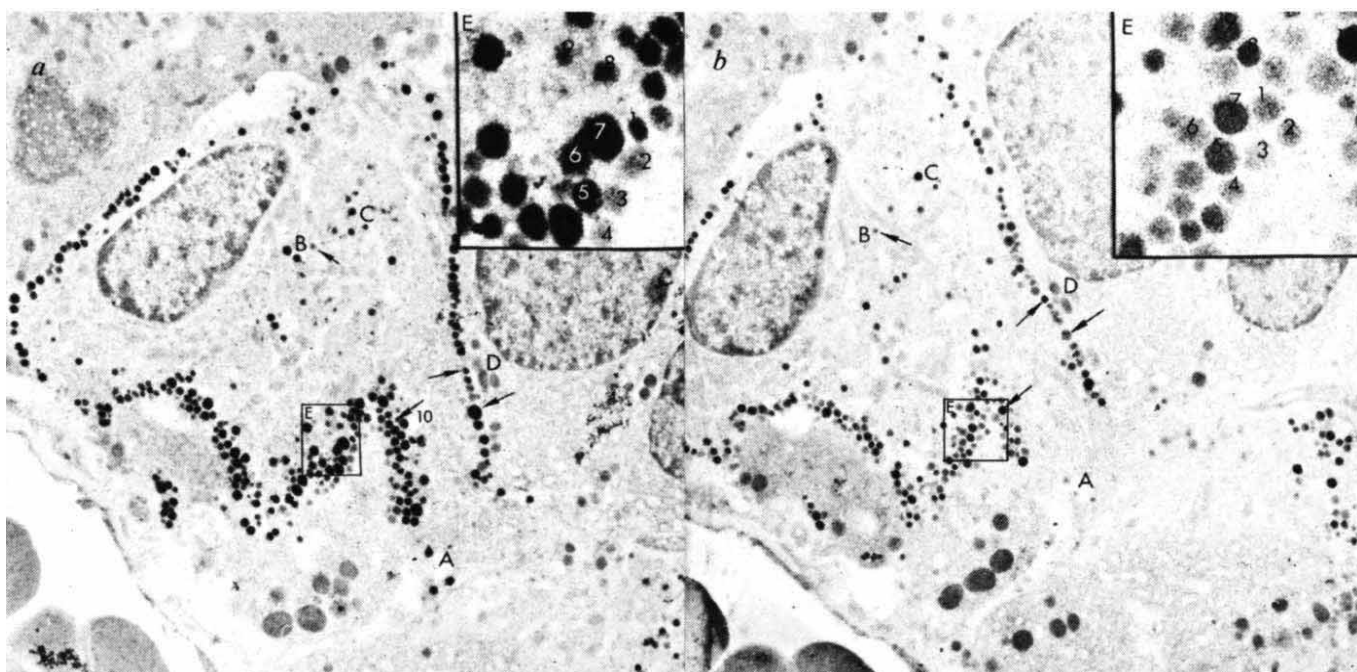


Fig. 2 *a*, Ultrathin section of female rat pituitary stained, immunocytochemically, with anti- ACTH_{17-39} serum. *b*, Section serial to that in *a*, stained with anti-rat FSH β . Cell A is stained with both antisera, although stain is weaker with anti-FSH β serum. The areas designated contain some of the same granules. Areas A and B show staining for ACTH on five granules. In area A of the cell stained for FSH β , granules are faintly visible. No stain is evident on them, nor on those in Area B. One granule in Area B stains for neither ACTH or FSH (arrow). Area C contains two granules which stain for ACTH and FSH β . Area D contains only one granule which stains for both hormones (arrow). The granule below it stains only for ACTH, as does the largest granule in the area (arrow). Area E was blown up as the inset. Granules 5, 7, 8, 9, and 10 stain for FSH β and ACTH. Granules 1 and 6 stain only for ACTH. Granules 2, 3, and 4 do not seem to contain either hormone. Granule 10 is not shown in the inset in *a*; it is designated by an arrow in *a* and *b*. Magnifications: $\times 4500$, inset $\times 20,000$.

Table 1 Densitometric analysis of specificity of the reaction with the anti-FSH β and the anti-ACTH sera

Antiserum*	Normalised staining intensity† of secretory granules
Anti-FSH β ‡	0.20 $\pm$ 0.002 s.e.m.
Anti-FSH β + 1 ng FSH ml ⁻¹	0.15 $\pm$ 0.01
Anti-FSH β + 10 ng FSH ml ⁻¹	0
Anti-FSH β + 10 μ g ACTH ml ⁻¹	0.18 $\pm$ 0.02
Anti-ACTH ₁₇₋₃₉ sera§	0.30 $\pm$ 0.01
Anti-ACTH ₁₇₋₃₉ sera + 10 ng ACTH ml ⁻¹	0.18 $\pm$ 0.008
Anti-ACTH ₁₇₋₃₉ sera + 100 ng ACTH ml ⁻¹	0
Anti-ACTH ₁₇₋₃₉ sera + 1 μ g FSH ml ⁻¹	0.32 $\pm$ 0.13

*1:2000 dilution anti FSH β , 1:5000 dilution anti-ACTH₁₇₋₃₉. Antigen added to 1 ml of diluted sera 2 d before its use in the stain.

†Normalised staining intensity values are optical density measurements of 50–75 granules per sample. Nuclear chromatin used as background to correct for section thickness 4.

‡Obtained from Dr A. F. Parlow, National Institutes Arthritis Digestive and Metabolic Diseases. FSH antigen-I-3 preparation.

§Produced in this laboratory. ACTH antigen-III World Standard.

There is little evidence for a functional relationship between FSH and ACTH. There is evidence for adrenal control of gonadal cycles⁹ and oestrogen stimulation of ACTH release^{10,11}. As discussed in a recent review⁹, however, there is more evidence for a functional link between ACTH and LH than ACTH and FSH. Yet our studies show that Type III LH cells are infrequent. They comprise only 4–7% of the total LH cell population, compared with 20–30% of the FSH cell population in cycling female rats⁷.

Recent studies by Pouplard *et al.*¹² have shown that human pituitary ACTH cells bind Fc fragments in a manner similar to that exhibited by mast cells and basophils. It is possible that the staining in rat ACTH cells represents a similar nonspecific affinity for immunoglobulin molecules. If this were the case, however, the controls in our study would have shown staining; for example, we would not have abolished staining by previous absorption with the antigen. Therefore, our tests show that the staining is more likely to be a specific immunochemical reaction for FSH and ACTH.

In conclusion, it seems that the old system of identification of pituitary cell types (based on granule size, distribution, cell shape) does not provide accurate information about a cell's hormone content. This is certainly true for the gonadotrophs^{6,7}, and as we have shown in this study, it is true for ACTH cells as well. One cannot distinguish an ACTH cell from an ACTH-FSH cell solely on the basis of morphology. We recognise the need for studies of animals in different physiological states before further conclusions are drawn. In fact, the relationship between the two hormones may become clearer as more animals in different physiological states are examined. We also recognise the need for further purification of the antisera.

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Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat

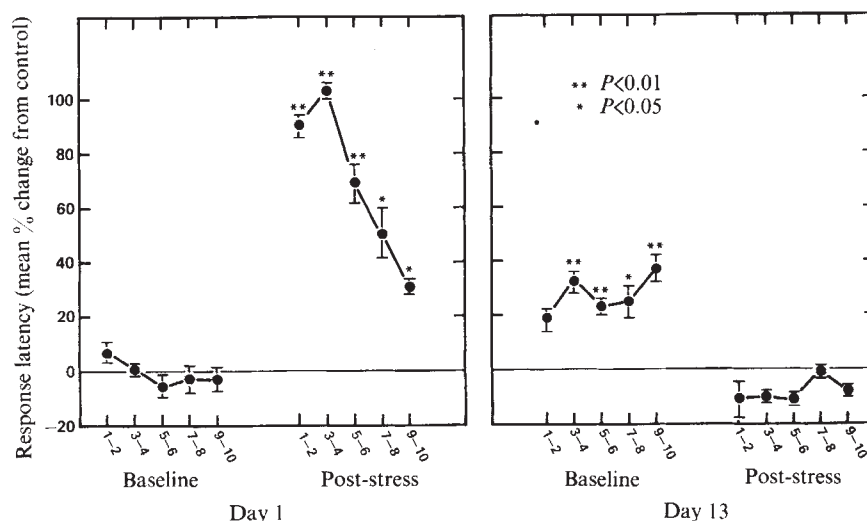
THE isolation and identification of endogenous opiate-like peptides from mammalian brain^{1,2}, and from pituitary^{3–5} has led to an active investigation of their pharmacological properties. These peptides—the enkephalins and endorphins—have been shown to produce naloxone-reversible analgesia^{6,7}, tolerance, and withdrawal⁷. While these effects seem consonant with those of morphine and other exogenous narcotics, little is yet known about the intrinsic, physiological functions of the opioid peptides. Since powerful analgesia is a prominent and reliable phenomenon associated with the administration of both narcotic alkaloids and brain opioid peptides, it seems reasonable to suggest that the opioid peptides may normally function to modulate pain responsiveness. Furthermore, work with stimulation-produced analgesia has suggested the existence, in mammalian brain, of a pain-inhibitory system with an opiate-like link^{8,9}. We have, therefore, hypothesised that such an endogenous system may be engaged by either environmental, sensory, or other physiological events which lead to adaptive changes in pain responsiveness. For example, noxious or stressful stimuli may recruit pain-inhibitory mechanisms in the central nervous system, bringing about alterations in enkephalins and endorphins, with concurrent or subsequent changes in responses to pain. Consistent with this hypothesis, we now report that inescapable acute stress causes a significant increase in levels of opioid peptides with a concurrent decrease in pain responsiveness in the rat. Furthermore, both the biochemical and behavioural changes are “reversed” by repeated exposure to stress. A preliminary report of this work has been made¹⁰.

To assess the effects of either acute or repeated exposure Sprague–Dawley rats (400–450 g) were initially tested in the tail-flick procedure on each of two occasions: immediately before the first 30 min stress session (day 1), to obtain a baseline latency, and immediately following this stress session. Thereafter, these experimental animals were re-exposed to the 30 min stress session, once daily, for a total of 13 consecutive sessions. On day 13 the animals were again tested in the tail-flick procedure for pain responsiveness on each of two occasions in the manner previously outlined. Comparable numbers of non-stress controls were, likewise, tested for pain responsiveness at appropriate times on both days 1 and 13.

Stress was produced by a 30-min period of intermittent, inescapable foot shock. During each session, rats were individually placed on the grid of an operant chamber, where a 3-mA scrambled shock stimulus was delivered for 1-s duration, every 5 s.

The pain responsiveness test was a modification of the tail-flick measure of D'Amour and Smith¹¹, and is described elsewhere¹². Briefly, it involves focusing radiant heat on the tip of the rat's tail, and measuring the latency of the withdrawal reflex. The radiant heat source was adjusted such that the majority of the animals responded between 3.5 and 4.0 s during baseline testing. A cut-off point of

Fig. 1 Effect of acute and repeated stress on tail-flick latencies. Pain responsiveness was measured before (baseline) and after 30 min of stress (post-stress) on days 1 and 13. Response latencies of experimental animals ($n = 4$) were transformed into percentage change from control ($n = 5$) for each time point. Each point and corresponding bar represents mean $\pm$ s.e. for each pair of successive trials. Asterisks indicate significant differences from corresponding control trials.



6 s was imposed to prevent tissue damage. Ten trials were conducted during each test session, with 60–75-s intervals between trials.

To determine the possible effect of stress on endogenous opioid peptides, the levels of these peptides were measured in three separate experiments employing 33 animals. Since the results of these studies were internally consistent, they were subsequently pooled. The opioid levels were determined at four different time points. The initial two points were obtained by killing animals either before an acute stress session in order to measure control basal levels, or immediately following that stress session. The remaining two time points involved animals which had been subject to the repeated stress paradigm. These animals were killed on day 13 either before the stress session, in order to measure their resting levels, or immediately following stress. Endogenous opiate-like activity was measured using a ^3H -naloxone membrane binding assay^{13,14}. A P_2 fraction was prepared from whole brain, and the peptides were then extracted by hypotonic lysis (10 mM Tris-HCl, pH 7.7, at 25 °C), boiled for 15 min, and centrifuged for 1 h at 100,000g. Extracts from 40 mg of tissue (wet weight) were added to the binding preparation, and the inhibition of stereospecific opiate binding measured. Since we did not separate enkephalins from possible larger peptides, endogenous opiate activity was expressed in units, with one unit being defined as the amount which yields 50% receptor occupancy. All samples were assayed in duplicate; duplicate pairs differed by less than 10%. The endogenous opiate activity was abolished by treatment with carboxypeptidases, or by preincubation at 37 °C in the whole brain

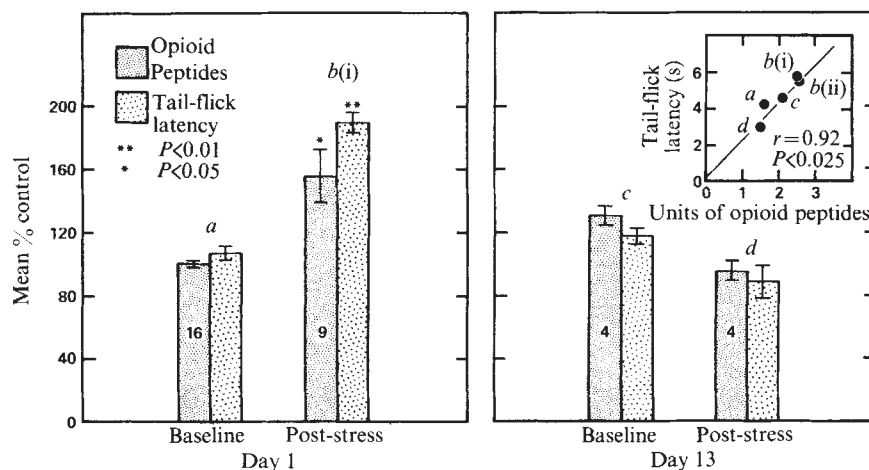
homogenates. Also, the effect of endogenous factors on stereospecific binding was enhanced by manganese and inhibited by sodium, indicating their specific and agonistic nature¹³.

Acute stress (30 min) results in a significant increase ($P < 0.001$) in tail-flick latency. As can be seen in Fig. 1 (day 1) analgesia is most dramatic during the first few trials immediately following foot-shock, with all experimental animals reaching the cut-off point (6-s response latency) on one or more of the initial trials. The pain responsiveness gradually recovers with successive trials. Nonetheless, these animals still remain significantly analgesic relative to non-stressed controls through the end of the testing.

Daily re-exposure to the 30-min stress session results in a significant elevation ($P < 0.001$) in baseline response latencies when tested before the stress session on day 13. However, the subsequent stress session does not induce any further analgesia. On the contrary, it brings about a hyperresponsiveness to noxious inputs, such that post-stress latencies are significantly shorter than their pre-stress baseline responses, and significantly shorter than the response latency of non-stress controls.

Figure 2 displays the parallel changes in opioid peptides and pain responsiveness measured at various times throughout the stress paradigm. Opioid peptides are significantly elevated after a 30-min acute stress session (day 1) when animals exhibit profound analgesia. Daily re-exposure results in moderately elevated resting opioid levels and an intermediate degree of analgesia on day 13. However, following stress on day 13, these levels decrease, returning to control values. Similar effects are obtained with 60 min

Fig. 2 Parallels between stress effects on pain responsiveness and opioid peptide levels. Opioid levels determined pre- and post-stress on days 1 and 13. Corresponding tail-flick latencies (trials 1 and 2 of each testing session, $n = 4$) for each time point are also displayed. Biochemical and behavioural data expressed as mean percentage control $\pm$ s.e. Number per group for opioid determination indicated at base of each bar. Inset demonstrates statistically significant correlation between units of brain opiate activity (per 40 mg wet weight) and tail-flick latencies (first two trials) under several stress conditions. *a*, Non-stress control. *b* (i), After 30 min acute stress; *b* (ii), after 60 min acute stress. *c*, Resting levels on day 13, after chronic 30 min stress. *d*, After 30 min repeated stress.



of acute stress, with experimental animals ($n=11$) exhibiting significant analgesia ($P<0.01$) and their brain opioid levels rising to 169% ($P<0.02$) of control levels.

The present results suggest a potential role of endogenous opiates in pain modulation and adaptive responses to stress. The levels of these peptides seem to rise with foot-shock stress, and this rise is accompanied by significant analgesia. This analgetic effect of acute stress has been independently described by other investigators¹⁵. While there is no *a priori* reason to attribute the full-blown analgesic effect of stress to the endogenous opiate system, it seems likely that this system may have an important role in the decrease in pain responsiveness observed after stress. Furthermore, repeated foot shock causes parallel changes in both opiate activity and behavioural responsiveness. Thus, the animals exhibit higher response latencies after chronic stress, and have slightly elevated resting levels of opiates. Yet further stress brings about a paradoxical decrease in response latencies, accompanied by a return of opiate levels to control values. It is apparent that the same stress has differential effects on behaviour and opioid levels, depending on the previous stress experience of the animal.

It should be noted that our biochemical measures involved overall opiate-like activity in the brain. While a large portion of this activity co-chromatographs with authentic enkephalins (Sephadex G-15, AG-1X2, 200–400, acetate form), it is quite possible that the larger brain opioid peptides recently described³ may also be involved in the effects we report. Further, it would be of interest to assess the possible role of pituitary opioids—not measured here—in the stress response. While the specific interactions between brain and pituitary opiates have not been determined, their interplay may prove critical for stress adaptation and survival.

The mechanisms underlying the present findings remain to be determined; however, it seems reasonable that both physiological adaptive mechanisms and learning mechanisms may be involved in the phenomena we have observed. The biochemical events leading to increased opioid levels could involve increased synthesis, decreased breakdown, or activation of previously inactive forms. Furthermore, it is possible that different brain regions exhibit differential effects of stress. In any case, it seems likely that the rise in levels is indicative of a potentiation of the endogenous opioid system, since we have demonstrated in a separate experiment¹⁰ that stress-induced analgesia is partially prevented by naloxone administration. This finding directly implicates opiate-receptor interactions in the change of pain responsiveness observed after stress. It therefore appears that the endogenous opiate peptides, by mediating some of the sensory changes that occur during stress, may provide an important interface between hormonal, sensory and emotional responses.

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Note added in proof: We have noted in subsequent experiments that, when enkephalins are separated from other opioid factors, specific enkephalin activity can exhibit changes which are different from those in overall opiate activity.

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Rapid eye movement cycle is a sleep-dependent rhythm

NORMAL nocturnal monophasic sleep is characterised by the cyclic alternation of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep, with the REM phase of this cycle recurring approximately every 90–110 min. Kleitman¹ hypothesised that an analogous 90–110 min basic rest-activity cycle (BRAC) exists during the awake state, implying a biological clock that continues to operate with the same periodicity throughout the 24-h period (that is, an activity-independent clock). We report here on the effect of altered sleep-wake schedules on the REM cycle and present evidence that the REM cycle is sleep-dependent—it operates only when the organism is sleeping and is not an expression of an activity-independent rhythm.

Several studies^{2–5} have supported Kleitman's BRAC hypothesis in identifying a 90–110-min cycle during waking in arousal level, perceptual illusions, gastric motility, and in some hormone secretions. It has been suggested^{2–4} that these cycles found in the awake state are expressions of the REM cycle rhythm in sleep. Under this activity-independent model, any wake time occurring within the REM cycle would not alter the REM cycle period; the time between successive stage REM onsets would remain constant, regardless of the amount of sleep or waking within the cycle.

Under the sleep-dependent model, wake time occurring between stage REM onsets should lengthen the REM cycle period, since the clock would stop on awakening. Brezinova^{6,7}, in studying the effects of spontaneous and externally imposed waking on sleep cycle length, reported that cycles interrupted by varying amounts of waking were significantly longer than intact cycles. Feinberg⁸ compared the sleep of elderly subjects, which is characterised by frequent and sometimes prolonged awakenings, with that of younger subjects. He found the REM cycles in the two groups virtually identical when the intervening wake time was subtracted. In addition, the first REM onset in normal nocturnal sleep is invariably preceded by 60–120 min of non-REM sleep, despite varying sleep onset times. These findings imply that it is not simply total elapsed time that determines cycle length, but sleep time.

Altered sleep-wake schedules such as in napping, or multiphasic sleep, provide an opportunity to test the sleep-dependent hypothesis (Fig. 1). We have analysed the sleep of 25 adults, aged 17–40, from three different sleep laboratories who underwent altered sleep-wake schedules. Following one baseline night of normal sleep, 8 subjects (Naval Health Research Center, San Diego)⁹, underwent a 60-min sleep–160-min wake schedule for 40 h. Ten subjects (Stanford University School of Medicine)¹⁰, had two baseline sleep nights followed by a 30-min sleep–60-min wake

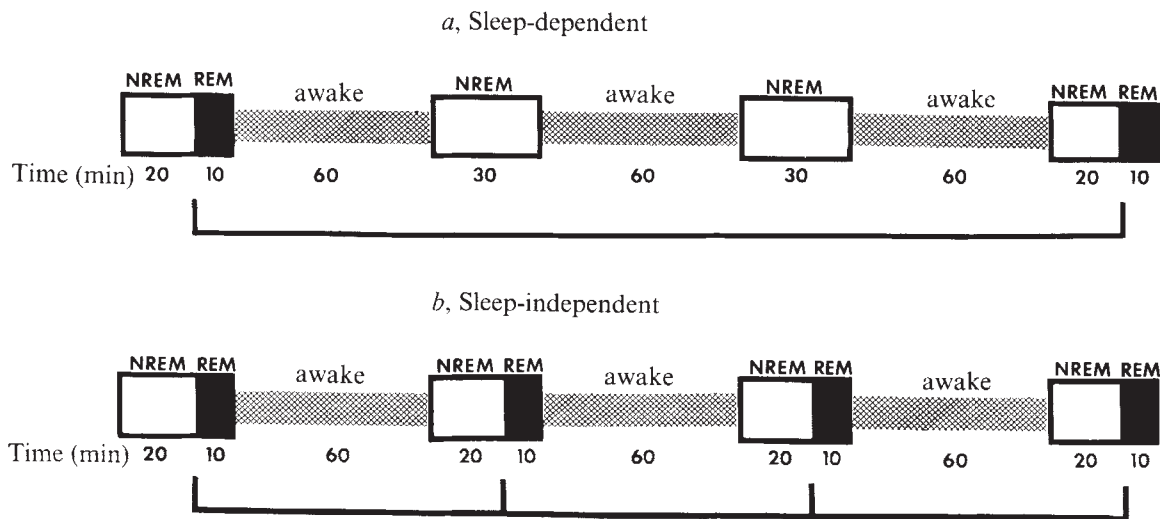


Fig. 1 Hypothetical examples of REM onsets occurring during a 30-min sleep—60-min wake schedule. *a*, Total sleep time between REM onsets is 90 min (total real time is 270 min). The 'clock' governing the time interval between REM onsets runs only during sleep; it stops on awakening and resumes at the next sleep onset. *b*, Total real time between REM onsets is 90 min (total sleep time is 30 min). The 'clock' runs during both sleep and waking. Studies of nap sleep from three independent sleep laboratories support the sleep-dependent model¹.

schedule for 5 d. Seven subjects (Montefiore Medical Center)¹¹, slept normally for 7 baseline nights and then were placed on a 60 min sleep–120 min wake cycle for 10 d.

If the REM cycle clock is sleep-dependent, stage REM onset should appear only after 90–110 min of sleep has

found in some waking activities is not an expression of the REM cycle found in sleep, but is a separate neurophysiological process, (2) the nap REM cycle period is not modulated by the circadian sleep–wake cycle, and (3) the REM cycle clock is sleep-dependent.

Table 1 REM cycle periods for nap and baseline sleep conditions

	San Diego N=8	Stanford N=10	Montefiore N=7
Nap RCP	96.6 (±20)	79.1 (±23)	119.2 (±30)
Baseline RCP	97.1 (±12)	92.0 (±12)	104.6 (±12)
Nap-Baseline RCP	−0.50 (±21)	−12.9 (±24)	14.6 (±31)
	Not significant	Not significant	Not significant

accrued since the last REM onset, regardless of the amount of wake time between or within the scheduled sleep periods. Using the last REM onset on the last baseline night as a starting point, we 'compressed' the sleep during naps for each group of subjects by subtracting all intervening wake time, and viewed this sleep as one continuous sleeping period. For both baseline and naps, a REM cycle period (RCP) was defined as the sleep time between two successive stage REM onsets.

According to the sleep-dependent hypothesis, the RCP in naps should not differ significantly from the baseline night RCP. We computed the average difference between nap and baseline RCP for each subject, and the overall average difference was tested for significant (0.05 level) deviation from zero with a *t* test. None of the mean differences differed significantly from zero (Table 1).

Was this sleep-dependent rhythm only present during a specific phase of the circadian sleep–wake cycle? To answer this question, we combined the 3 studies and compared nap REM cycles starting during the time period in which sleep usually occurs (2000–0800) with those cycles starting during the normal wake period (0800–2000). An equal number of naps occurred in the two 12-h periods. The REM cycle durations for the 0800–2000 and 2000–0800 time periods were 87.3 min and 88.6 min respectively, a non-significant difference.

These findings suggest that (1) the 90–110-min cycle

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Endorphin from pituitary inhibits cyclic AMP formation in homogenates of neuroblastoma X glioma hybrid cells

OPIATES inhibit basal and prostaglandin E_1 -stimulated formation of cyclic AMP by homogenates of cultured neuroblastoma X glioma hybrid cells^{1,2}. The opiate inhibition is stereospecific and is blocked by the specific opiate antagonist naloxone. Thus, interaction of an opiate agonist with its receptor inhibits adenylate cyclase activity.

Peptides with opioid activity (endorphins) have been found in the pituitary of several species^{3,4} and have been partially purified⁵. The pituitary peptide β -lipotropin (β -LPH) gives rise to biologically active fragments containing a pentapeptide sequence (methionine-enkephalin) with opioid activity at the NH_2 -terminus⁶, and this pentapeptide has also been found in brain⁷. In porcine pituitary, the principal opioid activity is associated with peptides that differ from all the active β -LPH fragments in that their biological activity is degraded by trypsin, as measured in the guinea pig myenteric plexus-longitudinal muscle bioassay⁸. Further characterisation of these endorphins is presented elsewhere⁹. Since endorphins derived from pituitary interact with opiate receptors in binding assays and have typical morphine-like actions in bioassays^{5,10,11} it was of interest to determine if this pituitary endorphin had typical opioid effects upon adenylate cyclase activity.

The source of the endorphin was the 'MSH concentrate' from porcine pituitary described elsewhere⁵. The guinea pig myenteric plexus-longitudinal muscle¹² was used to assay all fractions during purification. A 'guinea pig unit' (GU) is defined as the amount of material giving naloxone-reversible inhibition equivalent to that produced by 1 nmol of normorphine in the 5-ml tissue bath (that is, 200 nM final concentration). Half-inhibition of the muscle twitch amplitude requires approximately 0.5 nmol normorphine (100 nM). The crude material was first fractionated on a BioRex-70 cation exchanger (BioRad Labs., Richmond, Ca, column size 4 x 90 cm); activity was obtained in a basic peak eluting with 0.25 M ammonium carbonate. The active fraction was passed through a BioGel P-6 column (2.5 x 52 cm) in Tris-HCl buffer (50 mM) at pH 7.4; activity was found in a peak with apparent molecular weight about 3,000. This material was refractionated on BioGel P-6 in distilled water, in order to take advantage of adsorptive properties of the column for peptides at low ionic

strength. The activity peak emerging from this column had specific activity 132 GU/A₂₇₄ Unit, whereas the specific activity of the starting material was 7.2 GU/A₂₇₄. (A₂₇₄ Unit=amount of material in 1 ml solution with 1 cm light path giving absorbance 1.0 at 274 nm, the tyrosine extinction maximum.) The endorphin was concentrated on an Amicon UM05 filter (Amicon Corp., Lexington, Mass.) and portions were lyophilised and shipped in sealed tubes from Palo Alto to Bethesda.

This endorphin preparation was still impure, as evidenced by the presence of several ninhydrin spots on thin-layer chromatography plates developed in n-butanol:acetic acid:water (1:1:1, v/v/v). However, it is of interest because, as documented elsewhere⁹, it represents most of the opioid activity in the pituitary, it exhibits high potency, and it is clearly different from any active fragment of β -lipotropin.

The procedure used to measure adenylate cyclase activity in neuroblastoma X glioma NG108-15 hybrid cell homogenates was as described by Sharma *et al.*¹

Table 1 shows the effects of this pituitary endorphin on adenylate cyclase activity of the neuroblastoma X glioma NG108-15 hybrid cell homogenates. Endorphin was a more potent inhibitor of basal and PGE₁ stimulated adenylate cyclase activity than was morphine. Here the potency of the pituitary endorphin was 10 times that of morphine when the peptide concentration was calculated from absorbance at 274.5 nm, assuming one tyrosine per mole. Based upon normorphine equivalents in the guinea pig myenteric plexus bioassay, the potency inhibiting adenylate cyclase was 100 times that of morphine.

An experiment similar to that shown in Table 1 was performed also with a different endorphin preparation, containing material of about 1,750 daltons obtained from a commercial porcine crude ACTH preparation⁴. The results were virtually identical to those reported here for the 3000-dalton endorphin from MSH concentrate.

Fig. 1 shows the concentration-dependent blockade by naloxone of the inhibition of adenylate cyclase by endorphin in neuroblastoma X glioma cell homogenates. Complete antagonism of the endorphin effect could be achieved, except at the highest endorphin concentrations, and the curves are generally indicative of the expected competitive agonist-antagonist relationship. Using the method of Kosterlitz¹³, K_i for naloxone can be estimated as approximately 5×10^{-8} M, in reasonably good agreement with that obtained in this system with morphine (2×10^{-8} M) or methionine-enkephalin (3×10^{-8} M) (W. Klee and M. Nirenberg, unpublished observations).

Table 1 Effect of pituitary endorphin on adenylate cyclase of neuroblastoma X glioma cell homogenates

Addition		Cyclic AMP formed (pmol min ⁻¹ mg ⁻¹ protein) —Naloxone +Naloxone (10 μ M)		% of control —Naloxone +Naloxone (10 μ M)	
Basal	None	25	24	100	97
	Morphine (10 μ M)	14	23	56	95
	Endorphin (0.016 μ M)	18	24	72	96
	Endorphin (0.16 μ M)	13	23	52	92
	Endorphin (1.6 μ M)	11	18	44	72
+ PGE ₁ (0.25 μ M)	None	265	275	100	104
	Morphine (10 μ M)	192	262	72	99
	Endorphin (0.016 μ M)	263	269	99	102
	Endorphin (0.16 μ M)	212	272	80	103
	Endorphin (1.6 μ M)	174	256	66	97

Procedure of Sharma *et al.*¹. Incubations were for 5 min at 37 °C with the following components in a final volume of 100 μ l: 15 mM Tris-HCl (pH 7.5); 1 mM α -³²P-ATP, 10⁶ c.p.m.; 1 mM ³H-cyclic AMP, 10⁴ c.p.m.; 5 mM MgCl₂; 20 mM creatine phosphate; 0.5 mM RO20-1724 as cyclic AMP phosphodiesterase inhibitor (0.1% ethanol final concentration) creatine phosphokinase, 160 units mg⁻¹, 10 units; 0.16 M sucrose; 0.097 mg NG108-15 homogenate protein (frozen cells stored at -80 °C in 0.32 M sucrose, 0.01 M Tris-HCl, pH 7.5). Endorphin concentrations are normorphine equivalents, according to activity assayed in the guinea pig myenteric plexus-longitudinal muscle preparation, where normorphine and morphine have equivalent potencies. Data are means of duplicates from a representative experiment that was repeated three times.

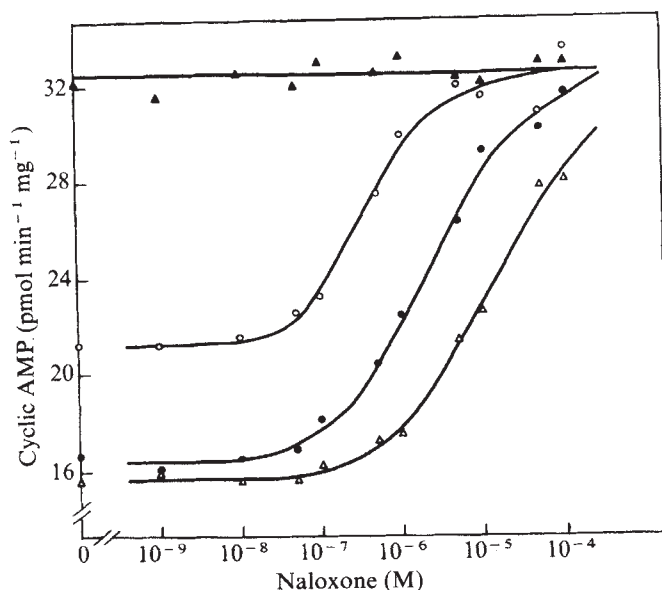


Fig. 1 Naloxone blockade of the endorphin inhibition of adenylate cyclase in neuroblastoma $\times$ glioma cell homogenates. Method as in Table 1. $\blacktriangle$, Control, no endorphin; $\circ$, 0.033 μ M endorphin (as normorphine equivalent in myenteric plexus-longitudinal muscle bioassay); $\bullet$, 0.33 μ M endorphin; $\triangle$, 3.3 μ M endorphin.

Our results indicate that pituitary endorphin has typical opioid effect on adenylate cyclase of neuroblastoma $\times$ glioma NG108-15 homogenates. Since opioid peptides of molecular weight in the 3,000-dalton range, possibly identical to pituitary endorphins, are found in brain¹⁴, it is likely that our findings have physiological significance for the function of endorphins in the nervous system.

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Feedback loop or output pathway in striato-nigral fibres?

THE turnover of dopamine has been thought to be controlled by a 'feedback' pathway from the postsynaptic dopamine receptors in the striatum to the dopamine-containing cells in the substantia nigra¹. This concept formed the basis of an explanation of the effects of neuroleptics on dopamine turnover^{1,3}. Evidence for a striato-nigral α -aminobutyrate (GABA)-containing pathway^{4,6} has supported the idea although there have been dissenting voices^{7,8}. In particular, the discovery of dopamine receptors on the dopamine-containing cells themselves^{9,10} has led to the suggestion that these nigral receptors might explain results otherwise explained by the feedback hypothesis⁸. Nevertheless, the existence of these receptors does not exclude the action of feedback loop in the control of dopamine turnover. We report here an attempt to evaluate the action of the striato-nigral pathway in the control of dopamine metabolism. Our results suggest that lesions in the GABA-containing striato-nigral pathway, which spare the dopamine-containing fibres of the nigro-striatal system, do not affect dopamine metabolism in the nigro-striatal dopamine neurones or the increase in dopamine turnover after administration of haloperidol.

Radioactive L-leucine, injected into the brain, is taken up rapidly by neuronal cell bodies in the region of the injection site, incorporated into proteins and transported along the axons of the cells¹¹. Output fibres from a given set of neurones can thus be traced autoradiographically¹² and after injections of 4,5,³H-L-leucine into the striatum the route followed by the striato-nigral fibres was traced in the brains of six albino Wistar rats. At the level of the hypothalamus shown in Fig. 1, the radioactivity marking the axons of this pathway is wholly contained within the internal capsule, well separated from the dopamine-containing fibres of the nigro-striatal system which have not entered the capsule at this level¹³.

An initial series of animals with 6-mC electrolytic lesions aimed at this area could be divided into two groups (Fig. 2). One group characterised by small well localised lesions showed a marked turning response towards the lesioned side with both apomorphine (0.5-5 mg kg⁻¹) and amphetamine (2 mg kg⁻¹) in spite of intact dopamine neurones assessed both biochemically and in the fluorescence microscope. The other group of rats showed no turning response and their lesions were located outside the internal capsule.

A further 12 rats showing the turning response to apomorphine after electrolytic lesions in the striato-nigral pathway were used to assess the effect of the lesion on the biochemical parameters of dopamine turnover. Using modifications (unpublished results of N.M.N.) of the published GLC methods¹⁴ we have measured the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) in striatum and of GABA in the substantia nigra. The striatal dopamine concentration was also measured by a sensitive radiochemical enzymatic assay¹⁵. Previous experiments have shown that GABA levels in nigra drop by 50% after hemisections of brain between striatum and nigra⁴. Table 1 shows that although the lesions were as effective as complete hemisections in reducing the concentration of GABA in nigra, the response of the dopamine system to haloperidol is not substantially different on the lesioned side from that on the intact side. Indeed there are no significant differences between the lesioned animals and normal animals in any of the measures of dopamine metabolism.

Published evidence¹⁶ and our own studies seem to indicate only two outputs from the striatal cells as seen by autoradiography. One goes direct to substantia nigra, and the

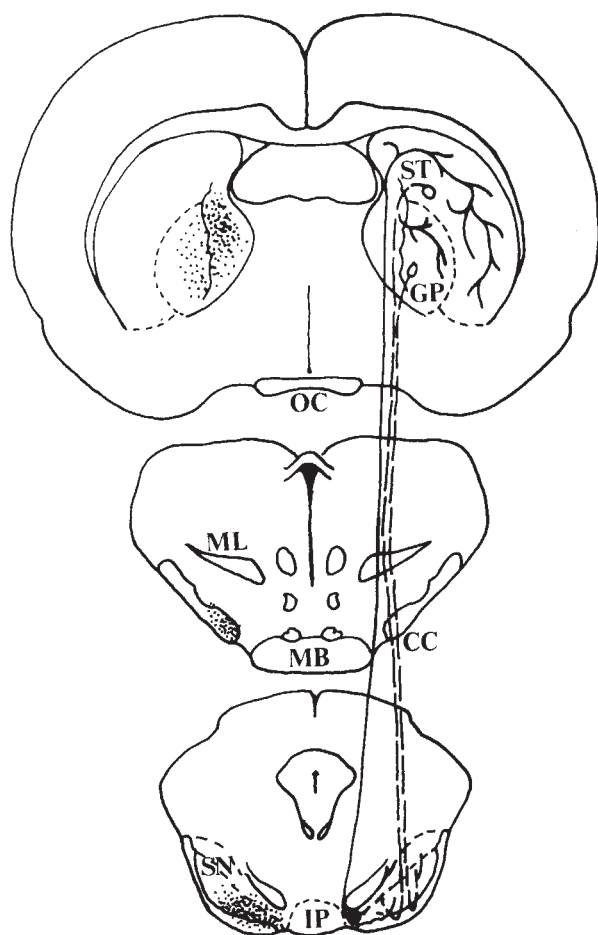


Fig. 1 The striato-nigral (GABA) and nigro-striatal (dopamine) system in rat brain. The dotted areas on the left of the sections summarise the distribution of autoradiographically localised silver grains 4 d after the injection of ^3H -leucine into the striatum. On the right are represented the pathways to and from striatum and substantia nigra reported in the literature^{13,17}. The solid lines and filled cell body represent the nigro-striatal dopamine system; the open circles and dotted lines represent the GABA containing striato-nigral systems. At the level of the mamillary bodies represented by the middle section the GABA fibres run separately from the DA ones which enter the internal capsule at a level anterior to this. GP, Globus pallidus; CC, crus cerebri; IP, interpeduncular nucleus; MB, mamillary bodies; ML, medial lemniscus; OC, optic chiasma; SN, substantia nigra, and ST, striatum.

other synapses in the globus pallidus. There are GABA-containing fibres projecting from pallidum to nigra¹⁶ but the pathway which they follow is very close to the site of our lesion and this, in conjunction with the fall in GABA levels, suggests that we have extensively damaged the documented pathways from striatum to nigra, with no effect

on the control of striatal dopamine metabolism in either normal animals or in animals treated with haloperidol.

It is conceivable that any remaining neurones of the striato-nigral or of the pallido-nigral pathways could have functionally compensated for the reduction of GABA content in the substantia nigra by an increase in their responsiveness to neuroleptics. In addition to such a pre-synaptic adaptation, the GABA receptors in substantia nigra could develop denervation supersensitivity and compensate for the damage resulting from the lesions. Further work is required to demonstrate these possible compensatory mechanisms in the striato-nigral systems. Our present interpretation is that the striato-nigral pathway is not necessary for control of striatal dopamine turnover.

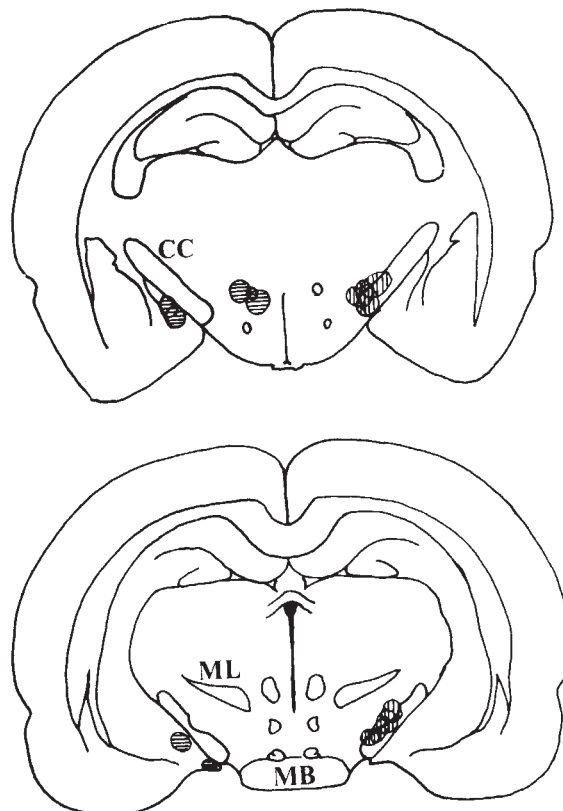


Fig. 2 Lesion sites from the first 14 rats projected on the coronal sections of the rat brain redrawn from ref. 22. The drawings represent sections 1 mm apart. The vertically-hatched lesion sites on the right are from eight rats which turned after apomorphine treatment (2 mg/kg i.p.). The horizontally hatched areas on the left represent the maximal extent of lesion sites in six animals which failed to show turning behaviour in response to the same dose of apomorphine. The abbreviations are those used in Fig. 1.

Table 1 Effect of striato-nigral pathway lesions on striatal dopamine metabolism and GABA concentrations in substantia nigra in untreated and haloperidol-treated rats

		GABA substantia nigra	Dopamine	Concentration in striatum HVA	DOPAC
Untreated animals	Lesioned side	117 ± 23 (4)*	9.4 ± 0.49 (8)	1.2 ± 0.11 (8)	1.1 ± 0.06 (8)
	Intact side	236 ± 54 (4)	9.2 ± 0.58 (8)	1.4 ± 0.13 (8)	1.2 ± 0.11 (8)
Lesioned animals 30 min after 1 mg per kg haloperidol	Lesioned side	147 ± 20 (8)*	5.2 ± 0.46 (8)†	4.6 ± 0.41 (8)†	3.3 ± 0.21 (8)†
	Intact side	319 ± 58 (8)	6.1 ± 0.53 (8)†	4.9 ± 0.51 (8)†	3.1 ± 0.2 (8)†

Concentrations are given as μg per g wet weight $\pm$ s.e. (n). 2–3 Weeks elapsed between lesion induction and the death of the animal. An area of brain including substantia nigra (SN) was dissected free hand on ice and frozen in liquid nitrogen 2–4 min after the death of the animal, when the post-mortem increase in GABA should have been complete²³. GABA concentrations in the table are uncorrected for recovery which was approximately 60%. Striata from the same brains were dissected on ice after the nigral dissection and frozen 4–8 min after decapitation. Estimates of HVA and DOPAC concentrations are also uncorrected for recovery but it was approximately 95%. Dopamine concentrations are corrected.

*GABA concentrations are significantly lower on the lesioned side, $P < 0.02$ (paired t test, two-tailed).

†These concentrations are all significantly different from the appropriate control values (in untreated animals), $P < 0.001$ (t test, two-tailed).

Speculation as to the function of the striato-nigral pathway is perhaps in order if it does not subserve the 'feedback' role usually designated to it. After induction of unilateral lesions in the nigro-striatal dopamine neurones with 6-hydroxydopamine, apomorphine in very low doses causes circling behaviour away from the lesioned side¹⁷. This behaviour is abolished by ipsilateral electrolytic lesions¹⁸ located in a similar site to that shown in Fig. 2. Animals with both nigro-striatal and the striato-nigral systems lesioned unilaterally show a turning behaviour similar to that reported after the striato-nigral lesions alone¹⁸. The turning behaviour seen after extensive unilateral ablations of the striatum is also similar in direction and drug sensitivity¹⁹. That is, the induction of unilateral lesions of the striato-nigral pathway, with or without damage to the dopamine neurones is equivalent to striatal ablation in its effect on turning behaviour. We have recently found deficits in passive avoidance behaviour in animals with bilateral lesions in the striato-nigral system, which is also similar to the deficits seen after large bilateral lesions²⁰.

Since the striato-nigral pathway terminates predominantly within the zone reticular region of substantia nigra it seems likely that it influences neural activity within this region. The recent electrophysiological evidence for a functional connection between striatum and thalamus through zona reticulata neurones²¹ suggests that the fibres which we have lesioned are a part of this system rather than a feedback loop controlling dopamine neurones in zona compacta of substantia nigra.

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Can chloropractolol alkylate β adrenoceptors?

EREZ *et al.*¹ have classified chloropractolol as an irreversible, covalently-binding, antagonist of cardiac β adrenoceptors. This conclusion was based on the knowledge that similar halogen amides can alkylate sulphhydryl groups^{2,3} and their observation that chloropractolol produced a long-lasting

antagonism of β adrenoceptors associated with a depression of the maximum response to isoprenaline.

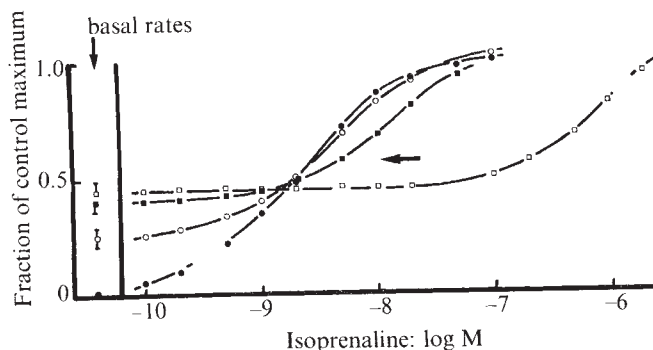
We have measured the contraction frequency of isolated rat atria, the system used by Erez *et al.*, and have found that ($\pm$) chloropractolol has agonist activity of its own with half-maximal response at 4×10^{-8} M ($n=7$) which is about five times the concentration for half-maximal response to ($\pm$) isoprenaline on this tissue. The increase in atrial frequency due to chloropractolol was not reduced by pre-treatment with reserpine but was promptly antagonised by ($\pm$) propranolol, 2×10^{-5} M. The maximum agonist response to chloropractolol, however, was 0.41 ± 0.02 ($n=8$) times the isoprenaline maximum (see control value in Fig. 1 for 10^{-5} M chloropractolol alone). This compares with a maximal response for ($\pm$) practolol of 0.26 ± 0.04 ($n=4$) times the isoprenaline maximum.

We have found that chloropractolol also has antagonist properties and therefore can be classified, like practolol⁴, as a partial agonist. Dextral displacement of the isoprenaline dose-response curve, produced after equilibration for 45 min with 10^{-5} M chloropractolol, is shown in Fig. 1.

The shift of the isoprenaline dose-response curves, or dose-ratio (DR), has to be estimated for a partial agonist from the difference in the logarithms of the concentration of isoprenaline producing half-maximal responses as described by van Rossum⁵. Dose ratios for isoprenaline were obtained after equilibration with chloropractolol concentrations of 10^{-8} to 10^{-5} M. A Schild plot of $\log (DR-1)$ against $\log [B]$, the chloropractolol concentration, gave a linear regression with a slope of 0.94 (95% confidence limits 0.88-1.0), which is not significantly different from unity. The estimated dissociation constant for chloropractolol was 1.2×10^{-8} M (95% confidence limits of 8.9×10^{-9} to 1.5×10^{-8} M). No difference in the apparent dissociation constant for chloropractolol was found when comparing atria from untreated rats or rats pretreated with reserpine. Our estimate of the apparent dissociation constant for practolol, based on similar experiments, was 9.8×10^{-8} M (95% confidence limits of 2.7×10^{-8} to 1.9×10^{-7} M) derived from a Schild plot of slope 1.2. The slope for practolol was not significantly different from that for chloropractolol.

In Fig. 1, the control log dose-response curve to isoprenaline has been normalised by expressing measured increases in atrial frequency as fractions of the maximal increase in frequency. The isoprenaline log dose-response curve

Fig. 1 Dose-response curves to isoprenaline in isolated rat atria. Ordinates: increase in atrial rate as a fraction of the maximal response to isoprenaline. Abscissae: log molar concentration of isoprenaline. The results are the average of four experiments. The curves shown are: isoprenaline alone ($\bullet$); repeated after equilibration with 10^{-5} M chloropractolol for 45 min ($\square$); repeated after four changes of bath fluid (30 ml) during 35 min ($\blacksquare$); and repeated after another six changes of bath fluid during 90 min ($\circ$). Basal rates to chloropractolol alone are shown separately.



obtained in the presence of the antagonist was found by expressing measured increases in atrial frequency produced by the combined agonist effects of chloropractolol plus isoprenaline as fractions of the control maximum increase in frequency due to isoprenaline alone. If, instead, the increases in frequency produced by chloropractolol are ignored and the results are presented as described by Erez *et al.* (where increases in atrial frequency due to isoprenaline in the presence of antagonist are expressed as fractions of the control isoprenaline maximal increase), then a family of curves is generated with progressively reduced maxima giving a spurious appearance of non-competitive antagonism. Erez *et al.* make no reference to agonist activity of chloropractolol and their dose-response curves may have been plotted using data normalised in a misleading way. Until this problem of the agonist effect of chloropractolol altering isoprenaline dose-response curves is solved, it might be prudent to put more weight on the time-course of effect as an indicator of 'irreversibility'.

An indication of the time course of recovery from blockade can be obtained from Fig. 1. The time course of the offset of antagonism can be followed by estimating $p = DR - 1/DR$ which is a function of fractional receptor occupancy, as defined by Paton⁶ and Schild⁷. In our experiments, dose ratios were measured from complete dose-response curves, so no more than three estimates could be made in each washout sequence. Nevertheless, plots of $\log p$ against time were close to linearity in each experiment, suggesting that first-order kinetics are an adequate description of the reversal of blockade. Rate constants for recovery from blockade, k_{off} , were calculated from these plots and their relation to initial dose ratio in each experiment is shown in Fig. 2. The slopes of the $\log (DR - 1)$ against $\log k_{off}$ regression lines for both chloropractolol and practolol are significantly different from zero; therefore k_{off} , which is apparently dependent on initial receptor occupancy, cannot be taken as an estimate of the dissociation rate constant for the drug-receptor complex. If the

tissue contained other high-affinity binding sites for these antagonists so that, during washout, the receptors could be supplied with drug by slow efflux from this other pool then a similar dependence of k_{off} on concentration would be found. Although the dissociation of the drug-receptor complex is unlikely to be the rate-limiting step in our system, estimated k_{off} is useful for comparing chloropractolol with practolol. Figure 2 shows that for equiactive concentrations of the antagonists, the k_{off} is significantly greater for chloropractolol than practolol and therefore there is no support here for an alkylation hypothesis. Depending on the initial concentration of antagonist the blockade washes out at a rate between 1% and 10% per min; no conclusion about alkylation can be based on this relatively slow washout because propranolol (10^{-8} M) washes out at a similar rate of 1-2% per min from isolated guinea pig atria⁸.

Thus, we have found that chloropractolol is a partial agonist at β adrenoreceptors with about 1.5 times the intrinsic activity and 10 times the affinity of practolol. We can find, however, no evidence that chloropractolol alkylates β adrenoreceptors in rat atrial muscle, and it is unlikely to be any more useful than other β adrenoreceptor antagonists as a tool in receptor isolation studies.

We thank Dr K. W. Bowden for synthesising chloropractolol. T.P.K. is a Canadian Medical Research Council Postdoctoral Fellow.

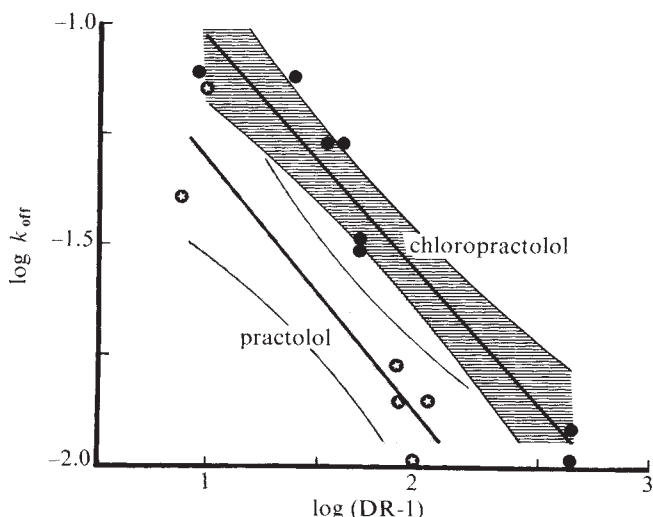
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Fig. 2 Time course of washout of antagonism of isoprenaline (for both chloropractolol and practolol) as a function of the initial degree of antagonism. This relationship has been linearised by expressing (on the abscissa) initial antagonism as $\log (DR - 1)$, as used in the Schild equation (where DR = dose ratio), and by expressing, on the ordinate, the rate constants for washout as $\log k_{off}$. Each rate constant was calculated from the apparent first-order kinetics shown by the linear regression of $\log p$ on time; p was calculated from the function $DR - 1/DR$. Analysis of variance showed that the slopes of both lines are significantly different from zero but not significantly different from each other ($t = 0.39$). Analysis of covariance showed that the data are best described by separate parallel straight lines; for vertical difference between values of Y at given x , $t = 5.52$.



Histamine blocking agent in the salivary gland homogenate of the tick *Rhipicephalus sanguineus sanguineus*

STUDIES of reactivity to tick bites and tick salivary gland homogenate have revealed responses similar to those induced by histamine in the skin of lop-eared rabbits¹. Salivary gland homogenates of the tick *Rhipicephalus sanguineus sanguineus*, however, did not cause contraction of guinea pig ileum, but blocked the action of histamine acid phosphate (HAP). We have therefore tested this extract against other drugs known to cause contraction of guinea pig ileum in order to discover the spectrum of its blocking effect on this tissue. We have found that the salivary gland homogenate of this tick antagonises and potentiates, respectively, the action of histamine and acetylcholine on guinea pig ileum.

Adult ticks at various stages of engorgement but never fully engorged were collected from dogs at a veterinary clinic and used within 18 h. They were washed in 1% merthiolate solution, dried on filter paper and stuck to the surface of low-melting-point (30 °C) wax by pressing the ventral surface down into the partially melted surface in a Petri dish and submerging the mouthparts and legs. The dish with the ticks was then placed in a deep freezer (-18 °C) for at least 1 h to harden the wax. The ticks were dissected in cold physiological saline (4 °C), and the

Table 1 Results of pharmacological tests on the salivary gland extract (SGE)

Substance	Concentration	Height of contraction of guinea pig ileum before administration of extract (mm)	Height of contraction of guinea pig ileum after administration of 0.4 ml SGE (mm)	Reduction in contraction of guinea pig ileum (mm)	% Increase or decrease (Time)
Histamine*	0.1 $\mu\text{g ml}^{-1}$	27.5	6.8	20.8	75.5
Histamine	1.0 $\mu\text{g ml}^{-1}$	37.0	23.0	14.0	37.84
Barium chloride	40 mM	44.0	35.0	9.0	20.51
Acetylcholine	0.1 $\mu\text{g ml}^{-1}$	25.0	31.5	6.5	26.0†

*Mean of data from two animals' tissues.

†There was an increase in contraction of the ileum following the application of acetylcholine (0.1 $\mu\text{g ml}^{-1}$) after the addition of the salivary gland extract (SGE).

glands were extracted and put into a thick sterilised watch glass containing cold physiological saline. The salivary glands were freed (as much as possible) of bits of tracheae, salivary ducts and other tissues. After washing in two changes of cold physiological saline, the glands were homogenised in the watch glass with cold physiological saline by means of a sterilised glass rod with a ground end. To each pool of 25 pairs of salivary glands 0.4 ml of cold physiological saline was added. The homogenate was then removed into a sterilised Bijou bottle which was swirled gently to mix the contents. The dissection of the ticks and handling of the glands and homogenate was as far as possible carried out in sterile conditions. The homogenate was used as soon as possible.

Pharmacological testing of the salivary gland homogenate was carried out as described by Perry⁵, on guinea pig ileum preparation in an organ bath containing Krebs solution at 37 °C aerated with a mixture of 95% oxygen and 5% carbon dioxide. Isotonic contractions were recorded on a physiograph (Narco Bio-Systems, Houston) using a microdisplacement transducer. Responses of the ileum to histamine acid phosphate (0.1 $\mu\text{g ml}^{-1}$, 1.0 $\mu\text{g ml}^{-1}$), acetylcholine (0.1 $\mu\text{g ml}^{-1}$) and 40 mM barium chloride were tested. The effects of the homogenate alone and against the action of histamine, acetylcholine and barium chloride on guinea pig ileum were also tested.

As before³, 0.4 ml of salivary gland homogenate itself did not contract guinea pig ileum. But it antagonised the contraction of guinea pig ileum preparation caused by HAP (0.1 and 1.0 $\mu\text{g ml}^{-1}$) by 75.5 and 37.84%, respectively. Similarly, 0.2 ml of the homogenate reduced the contraction caused by HAP (0.1 $\mu\text{g ml}^{-1}$) by 41.8%. The first test was reproducible (Table 1). The blocking action of the homogenate persisted for some time because the ileum did not contract after the homogenate had been washed off twice with Krebs solution and more HAP (0.1 $\mu\text{g ml}^{-1}$) had been added. This indicates that the extract binds firmly to the tissues of the guinea pig ileum. After 2 h of washing, however, HAP (1.0 $\mu\text{g ml}^{-1}$) produced a contraction of 35 mm. After removal of this and addition of HAP (0.1 $\mu\text{g ml}^{-1}$) there was a contraction of 30 mm which was comparable to the original contraction of 27.5 mm due to the same concentration of HAP.

We showed that the agent in the tick's salivary gland homogenate was not an effective general blocking agent by its inability to antagonise substantially the contraction of the guinea pig ileum caused by addition of 40 mM barium chloride. On the contrary, the homogenate potentiated by 26% the contraction caused by acetylcholine (0.1 $\mu\text{g ml}^{-1}$).

The pharmacological test showed conclusively that the salivary gland of *R. s. sanguineus* contains a histamine blocking agent. This emphasises the conclusion that the pharmacologically active substance detected in the saliva

of this tick and that of *Haemaphysalis spinigera*² is either histamine or closely related to histamine.

The advantage to ixodid ticks in having both a histamine-blocking agent and histamine or histamine-like substance in their saliva is clear. Ixodid ticks attach to their hosts for a relatively long time and so in the absence of a histamine-blocking agent in the ticks' saliva far more pharmacologically active substance would be released into the host's tissues than necessary.

Excessive amounts of histamine in the host's tissues will endanger the life of the host to the ultimate disadvantage of the tick. On the other hand, the presence of a histamine-blocking agent in the tick's saliva implies an efficient mechanism for regulating the amount of histamine in the host's tissues at any particular moment to the advantage of the tick; perhaps small amounts being released during the first period of slow engorgement and larger amounts being released during the final period of rapid engorgement when such amounts may be required to cause a more intense reaction in the host, leading to the extravasation of a large quantity of blood.

The potentiating effects of the salivary gland extract on the action of acetylcholine on guinea pig ileum suggests that this extract would potentiate the effect of acetylcholine at the neuromuscular junction. If this effect becomes excessive at the motor end plate, it would lead to motor paralysis, which has been observed both in humans and animals after attachment and engorgement of some species of ixodid ticks^{1,3}. Motor paralysis after the attachment of *Dermacentor andersoni* has been shown to be due to blockade at the neuromuscular junction^{4,6}.

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Mutual repression of synaptic efficacy by pairs of foreign nerves innervating frog skeletal muscle

CONSIDERABLE attention has been directed recently at the proposed phenomenon of synaptic repression. In lower vertebrates experimentally crossed nerves will successfully innervate inappropriate muscles; in certain cases, it has been observed that some fibres later regrow to their original muscles, where they form functional synapses. In some instances where this occurs, the foreign innervation then seems to lose effectiveness in making the muscle contract¹⁻³. Mark and his collaborators have concluded that repressed synapses of the foreign nerve remain morphologically intact⁴⁻⁶ and are capable of being reactivated rapidly if the original nerve is again cut⁷⁻⁹. Although other workers^{10,11} have shown persistent double innervation without apparent repression in the same or comparable preparations in fish, Yip and Dennis¹² have recently confirmed the existence of quickly reversible synaptic repression in salamander limbs. There have been suggestions that similar synaptic repression might exist in the mammalian central nervous system¹³. If this is confirmed, it could constitute an important mechanism for plasticity during development or experience^{14,15}. Thus it is important to determine the mechanism of repression and to assess the reasons for the variability in the degree of competitive interaction observed in different preparations.

Yip and Dennis¹², supporting the observations of Mark⁹, report a sharp drop in the quantal content of foreign nerve EPPs in salamander muscle, but no change in miniature EPP (mEPP) size. In that preparation, as in the fish muscles used, both foreign and original nerves form distributed endings along much of the length of the muscle fibres, making it difficult to identify and study the properties of individual nerve endings. In the experiments described below we find evidence of mutual repression of synaptic effectiveness by two foreign nerves innervating frog skeletal muscle, where synapses are located far apart on muscle fibres and individual synapses can be examined. In these cases the competitive interaction must be mediated somehow through the muscle fibres. The drop in efficacy takes the same form: lower quantal content without any clear-cut change in postsynaptic sensitivity to transmitter.

Sartorius muscles were transplanted to the lymph sac beneath the skin of the back of 3-4-inch bullfrogs (*Rana catesbeiana*) and sutured at normal rest length to the fascia of the back. One or two somatic motor nerves (spinal nerves IV and/or V) were implanted into the denervated sartorius muscle. These are mixed nerves, but those fibres forming junctions had conduction velocities characteristic of fast nerves. At different times after innervation muscles were removed to a bath of 15-18° normal frog Ringers, where measurements were made of synaptic potentials and of the contraction tensions elicited by a single stimulus (twitch) and short trains of 30-40 s⁻¹ stimuli (tetanus).

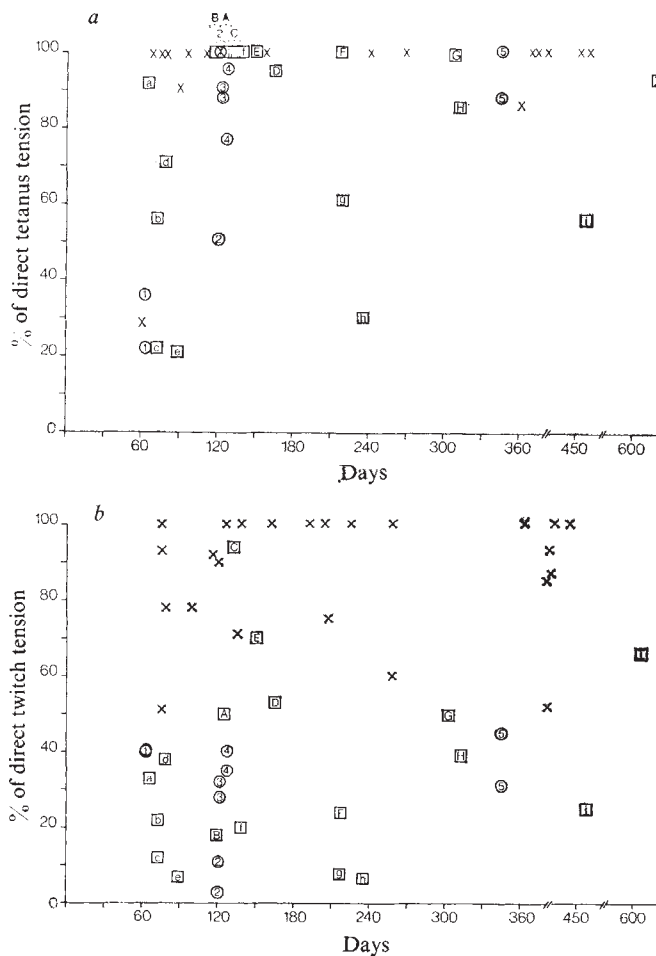
When a single foreign somatic motor nerve was implanted into the muscle, it rapidly innervated most or all of the fibres in the muscle (Fig. 1a). Within 2 months, tetanic stimulation of the nerve produced 90-100% of the maximal tension that could be elicited by direct stimulation of the muscle with an electrode applied across the pelvic end (98% ± 5% s.d., *n* = 17 at innervation times greater than 2 months). Maximal direct tension is taken to be that produced when all fibres are contracting.

When two spinal nerves were implanted simultaneously, both nerves seemed to innervate most fibres in the muscle; that is, each nerve was capable of driving the muscle approximately equally well, and in most cases both elicited tetanic tensions within 80% of the maximal direct tetanus

tension. Even when one nerve was implanted two or more months before the other, and would be expected to have innervated virtually all of the muscle fibres, the second was frequently able to drive many or all fibres as well (a,d,f). In other cases the second nerve was less effective (b,g,h,i), but succeeded in innervating many fibres which had presumably already been innervated by the first. The least effective nerves, c and e, had been implanted for relatively short times. In all cases the first nerve retained effective innervation on essentially all muscle fibres as judged by tetanic stimulation. The interpretation of these tension results as representing extensive dual innervation might be incorrect if strong contraction of one population of fibres could have mechanically induced antidromic action potentials in terminals of the other nerve, innervating a different population. However, no activity could be recorded on one nerve while tetanically stimulating the other, or while strongly stretching the muscle. Moreover, as will be described below, intracellular recording did show double innervation in a large fraction of the fibres.

These results indicate that the presence of one foreign nerve innervating a muscle fibre does not preclude innervation by a second. However, if synaptic effectiveness is judged by the ratio of indirect-direct twitch tension there is evidence for mutual synaptic repression (Fig. 1b). A

Fig. 1 Success of innervation by foreign nerves innervating transplanted sartorius muscles, as judged by measurements of the tetanic (a) and twitch (b) tensions. The tension produced by each nerve, calculated as a percentage of the maximum tension elicited by direct stimulation of the muscle, is plotted at different durations of innervation. ×, indicate success of innervation in singly reinnervated muscles. The encircled numbers represent 5 experiments in which 2 foreign nerves were implanted simultaneously. The letters inside the squares denote data from 9 experiments in which 1 nerve (capital letters) was implanted 2-3 months before the other (lower case letters).



single stimulus to either of two nerves implanted simultaneously caused only 20–50% of the fibres to twitch (mean = $31 \pm 14\%$ s.d., $n=10$). This was in marked contrast to the effectiveness of a single nerve, which, when implanted alone for 75 d or more (crosses in Fig. 1b), caused twitches in 70–100% of the fibres ($88 \pm 16\%$ s.d., $n=24$). The effectiveness of a nerve in producing a twitch was reduced even if it was implanted 2–3 months before the second, and was presumably able to drive virtually all of the fibres above threshold at the time when the second nerve was implanted. The proportion of fibres twitching to stimulation of the first nerve was $51 \pm 24\%$ in nine preparations, far less than if it had been implanted alone. The proportion twitching to the second nerve was generally smaller ($19 \pm 12\%$ s.d., $n=9$). That a high proportion of junctions had subthreshold responses was also evidenced by the large tetanus–twitch ratio for each of a pair of nerves (average 6.22 ± 4.3 s.d., $n=27$) compared with single reinnervated muscles (2.4 ± 1 , $n=21$).

Fibres that were strongly innervated by one nerve tended to be only weakly innervated by the other. Even when one nerve was implanted months before the other, each nerve seemed to have its own separate 'twitch field'. This could be seen both on visual inspection and from the observation that twitch tensions summated with little indication of overlapping innervation. Measuring overlap of functional innervation as the sum of the twitch (or tetanus) tensions to each nerve, minus the tension when both were stimulated simultaneously, divided by the smaller of the individual tensions^{16,17}, there was usually no more than 10–20% tension overlap of twitch populations, compared with 85–100% overlap of the populations driven by tetanic stimulation (Table 1). The arithmetic sum of the tensions produced by single stimulation of each of the two nerves, implanted simultaneously or at 2–3-month intervals (mean $67 \pm 25\%$, $n=14$), was approximately the same as the twitch tension to simultaneous stimulation of the two nerves ($64 \pm 23\%$, $n=14$) and both values were clearly smaller than the average twitch tension of singly innervated muscles ($88 \pm 16\%$, $n=24$).

As these tension measurements would predict, a large proportion of the EPPs recorded in doubly innervated muscles were subthreshold to a single stimulus. Moreover, the two nerves apparently seldom form terminals close together on muscle fibres. In approximately 80% of the fibres studied to date in which an EPP was found to stimulation of one nerve (170 of 215 fibres), the other nerve did not produce a recordable EPP at that site. The second nerve either elicited an action potential at some distant point or gave no apparent response. Figure 2a is a characteristic example of this group of muscle fibres. In the 20% of the fibres in which EPPs to both nerves could be seen at the same site they usually differed conspicuously in time course and amplitude, although the larger was not

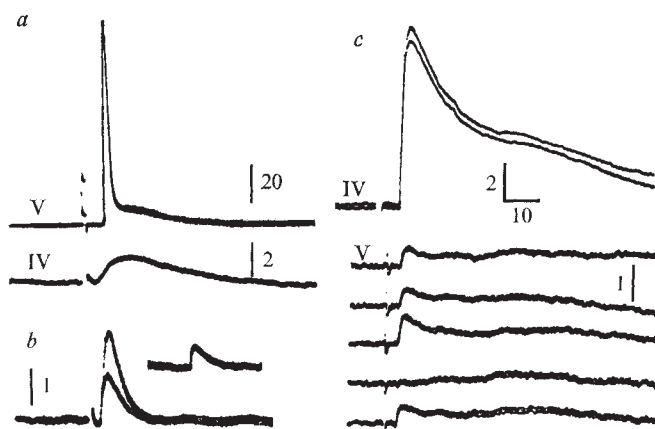


Fig. 2 EPPs from doubly-innervated muscles, all recorded in normal frog Ringer. *a*, Characteristic records from a fibre in a muscle innervated 118 days by both the IV and V spinal roots. At this recording site, nerve IV produced a small EPP, nerve V elicited a spike at some distant point in the fibre. *b*, From the same preparation, low quantal content EPP responses to two nerve V stimuli, compared with a miniature EPP from the same recording site. *c*, EPP responses to stimulation of nerve IV (superimposed top traces) and nerve V (lower series of traces) from a single recording site in a preparation innervated 320 d by nerve V, 259 d by nerve IV. Nerve IV elicited a large subthreshold EPP, nerve V a very low quantal content response that occasionally showed failures. Calibrations are in mV and ms.

always the one with the fastest time course. In less than 5% of fibres were both EPPs of comparable time course, suggesting that they could have arisen close together. We conclude that on most fibres the nerves end at considerable distance from one another, and that one nerve tends to produce a much stronger synaptic response than the other.

EPPs from the weaker synapses (for example, Fig. 2c, lower traces) were typically subthreshold in normal frog Ringer and showed quantal contents of 10 or less, judged by the amplitude variations of the EPPs and the ratio of average EPP to mean mEPP amplitude. This is in contrast to the EPPs at normal sartorius junctions¹⁸ or to those in singly reinnervated muscles, where virtually all are suprathreshold with quantal contents of 75–100 or more. The dominant synapses on doubly innervated fibres were usually, but not always, suprathreshold also. Their quantal contents, therefore, probably ranged from well above to just below the number required to exceed threshold (approximately 40–50). At the weaker synapses, mEPPs were infrequent, but had rise times and mean amplitudes (range 0.3–1 mV) characteristic of control neuromuscular junctions. Figure 2b shows an example. The reduction in synaptic efficacy was thus primarily due to a change in amount of transmitter released, without any evidence for a change in postsynaptic sensitivity. Repression of foreign neuromuscular junctions in salamander limb reportedly takes the same form^{9,12}. The reason for the lower-than-normal quantal content at certain synapses is not known but it apparently results from the influence of the other nerve innervating that fibre, and this influence can be exerted at such a distance that it must be mediated somehow through the muscle fibre.

Although the interaction observed here was between two foreign nerves rather than between the 'correct' (original) nerve and a foreign nerve, similar mechanisms may prevail in preparations showing more specificity. Indeed, although Scott⁶ has concluded that there is no displacement of a foreign nerve by the original nerve in fish extraocular muscles, she too saw a diminution of effectiveness of both foreign and original nerves, stimulated tetanically, compared with a single reinnervating nerve.

It will clearly be of interest to learn more about the

Table 1 Overlap of innervation by pairs of nerves

Preparation	Duration of innervation	Overlap % (twitch)	Overlap % (tetanus)
1	64, 64	16	30
2	121, 121	0	100
3	113, 113	15	85
4	126, 126	10	95
5	356, 356	12	100
A, a	125, 65	100	100
B, b	118, 72	0	100
C, c	132, 72	50	100
D, d	166, 79	13	90
E, e	50, 188	0	100
F, f	218, 139	8	100
G, g	307, 217	6	88
H, h	313, 237	0	85
I, i	620, 458	0	85

nature of the competitive interaction, the morphological distribution of axons from the two nerves, the inter-relationships of their terminals with each other and with old endplate structures, the mechanism(s) whereby one loses influence when a second nerve grows into the muscle, and the degree to which an original (appropriate) nerve is favoured in the competition.

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Is there retrograde axonal transport of tetanus toxin in both α and γ fibres?

It has been demonstrated by means of histoautoradiography that tetanus toxin, after injection into a muscle, reaches motoneurons in the spinal cord by retrograde axonal transport^{1–3}. It was until now not known whether the labelled motor axons and motoneurons belong to the α , the γ , or to both α and γ populations. We now present evidence that tetanus toxin is selectively transported in α motor neurons.

In two cats, ¹²⁵I-labelled tetanus toxin was injected into one gastrocnemius muscle (4 and 5 μ g toxin per kg body weight respectively; the toxin was labelled according to the method of Habermann^{4,5}, and contained 2 μ Ci and 4,500 mouse MLD. One cat MLD was equal to 5.4 μ g toxin/kg body weight). Three days later the cats were anaesthetised with Nembutal, a laminectomy was performed and the ventral roots L7 and S1 were ligated on the toxin side. The cats survived for another 8½ and 3½ hours respectively, during which time they were given intensive care⁶. The ligated roots were then excised and immersed in Karnovsky's fixative. Paraffin sections (8 μ m) were cut proximal and distal to the ligature and submitted to histoautoradiography using Ilford G5 emulsion. The autoradiographs were developed with Kodak D 19b developer after 71 d and 81 d of exposure at 4 °C. Outer myelin sheath circumferences were measured at a magnification of 800 with a Kontron Manual Optic Point Counting Device (MOP/AM 01). Fibre size histograms were constructed for a representative sample of all fibres (labelled and unlabelled), and for all the labelled fibres, in each of three ventral roots. An example is given in Fig. 1. Two populations of fibres were found, with peaks at around 5 μ m and 14 μ m diameter. These values are very similar

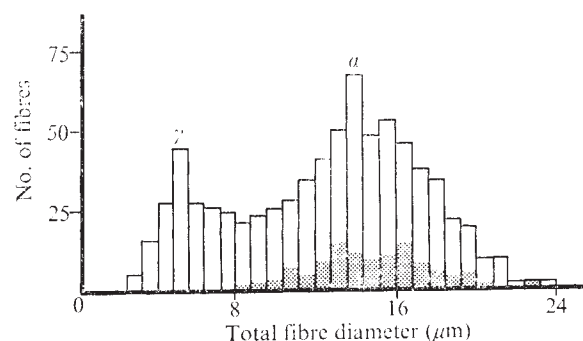


Fig. 1 Fibre size histogram from one fascicle of the ventral root L7 in a cat after injection of ¹²⁵I-labelled tetanus toxin into the ipsilateral gastrocnemius muscle. Open = labelled and unlabelled fibres ($n=742$), stippled = labelled fibres ($n=106$). n = Number of fibres measured. Total number of fibres in fascicle, ~1,500. Total number of labelled fibres, 106.

to those described previously for the γ and α populations of cat gastrocnemius motor fibres⁷.

The accumulation of radioactivity at the ligature would be expected to reveal the presence even of very small amounts of intra-axonal label. Even in these conditions, radioactivity in the ventral roots was found only in fibres which, from their size, seem to belong to the α population⁷.

This result is an interesting contrast to the findings of Strick *et al.*⁸ who injected horseradish peroxidase into cat gastrocnemius muscle and found γ -motoneurons even more intensely labelled than α -motoneurons. Further studies are needed into the mechanism responsible for the apparent lack of tetanus toxin in the γ axons. A barrier function of structural elements of the muscle spindle or a specific feature of the γ -fibre terminal must be taken into consideration.

Moreover, the increase of γ bias in local tetanus described by Takano *et al.*^{9,10} cannot be explained by assuming that tetanus toxin accumulates in γ motoneurons by intra-axonal ascent in γ -motor fibres.

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Modifications of nitrogen-fixing algae in lichen symbioses

THE factors responsible for the establishment of lichen symbioses involving nitrogen-fixing blue-green algae are poorly understood^{1,2} and it has not been clear how the algae are modified to remain in association, virtually ceasing to grow and yet fixing N₂ at high rates. We report here that in the lichen genus *Peltigera* morphological, physiological and biochemical modifications of the algae may all be involved in this process.

Comparison of symbiotic and free-living *Nostoc* spp. by light and electron microscopy showed that with *P. aphthosa*, which also contains a green alga (*Coccomyxa* sp.) and a fungus (an ascomycete) as symbiotic partners, the heterocyst frequency of the symbiotic *Nostoc* was 21% while that of the free-living *Nostoc* was 4–5%. The symbiotic algae of *P. canina* and *P. polydactyla*, which have a fungus only as partner, showed no increase in heterocyst frequency (remaining at 4–5% when symbiotic), probably because without another photosynthetic partner overall algal metabolism could not be sustained if more than a fifth of the cells were converted to non-photosynthesising heterocysts. The nitrogenase activity (acetylene reduction³) of the symbiotic alga of *P. canina* likewise resembled that of the free-living alga (6–10 nmol C₂H₄ per µg chlorophyll *a* per h) but the nitrogenase activity of the symbiotic alga of *P. aphthosa* (21 nmol C₂H₄ per µg chlorophyll *a* per h) was higher than that of the free-living phycobiont^{4,5} and could be accounted for in part at least by the increased frequency of heterocysts. There was also evidence that, in spite of the sustained nitrogenase activity, the symbiotic blue-green alga of each lichen was N-depleted. Thus, compared with free-living algae which accumulate large numbers of cyanophycin granules in post-log-phase growth^{6,7}, the symbiotic phycobionts showed no large scale deposition of such N reserves.

Physiological differences between free-living and symbiotic algae of *P. canina* were noted when the effect of combined N was investigated. Thus Table 1 shows that, as with other free-living algae⁸, NH₃ rapidly inhibited nitrogenase activity by the free-living alga, but had substantially less effect on the nitrogenase activity of the symbiotic alga, although an excess of NH₃ was available. Added NO₃⁻ did not inhibit nitrogenase activity by the symbiotic alga but partially inhibited that of the free-living alga. That is, in symbiotic algae but not in the free-living algae there is a mechanism which alleviates the inhibitory effect of combined N on nitrogenase.

The activities of important NH₃-assimilating enzymes in free-living and symbiotic algae were then examined. Attention was paid to glutamine synthetase (GS) which may be involved in the regulation of nitrogenase synthesis in heterotrophic bacteria^{9,10} and which is the key NH₃-assimilating enzyme in heterocystous blue-green algae¹¹, to glutamic dehydrogenase (GDH), the primary NH₃-assimilating enzyme in certain eukaryotes, including fungi¹², and to alanine dehydrogenase (ADH) and glutamate-aspartate-aminotransferase (GOT) which occur both in eukaryotic and prokaryotic algae. As Table 2 shows, when the *Nostoc* of *P. canina* was grown free from the lichen, GS activity was particularly high, GDH activity was negligible and GOT and ADH activities were appreciable. In the symbiotic alga, however, GS activity was reduced by 94%, GOT and ADH activities also decreased, and low GDH activities were measurable. In the intact lichen thallus which is mainly fungal hyphae, GDH activity was very high and GS activity was negligible. Possibly the low activities of GDH associated with the symbiotic algae were due to contaminating fungal hyphae in this fraction. In the

cephalodia of *P. aphthosa* which consist of *Nostoc* cells and intertwined fungal hyphae, GS activity was much lower than in the remainder of the thallus, indicative of low GS activity in the blue-green algae, but cephalodial GDH activity was much higher than in the remainder of the thallus. Thus, when the blue-green algae develop symbiotically a substantial decrease in activity of their key NH₃-assimilating

Table 1 Effect of NH₄Cl and KNO₃ on acetylene reduction by disks and by the isolated *Nostoc* of *Peltigera canina*

Concentration of NH ₄ Cl or KNO ₃ (mM)	C ₂ H ₂ reduction after 24 h (% original rate)			
	NH ₄ Cl		KNO ₃	
	Disks	<i>Nostoc</i>	Disks	<i>Nostoc</i>
0	107	110	107	113
1	58	3	95	42
2	54	3	97	51

The material (disks, 20 × 1.0 cm in diameter, in 50 ml of culture medium¹⁷) or 50 ml of log phase *Nostoc* were incubated with stirring at 3000 lx and 20 °C for 24 h in the presence of various concentrations of combined N. Acetylene reduction was assayed immediately before addition of the combined N and 24 h later. The level of exogenous combined N (refs 18, 19) did not fall below 0.5 mM and 1.4 mM in the 1-mM and 2-mM cultures, respectively. The initial activities observed were: disks, 7.4 nmol C₂H₄ per µg chlorophyll *a* per h; free-living *Nostoc*, 9.4 nmol C₂H₄ per µg chlorophyll *a* per h. Each value is the mean of triplicate determinations.

enzyme GS occurs and in the absence of effective levels of GDH this may result in N-limitation of the algae, in an inability of the algae to grow normally, and in a reduction of the effects of NH₃ and NO₃⁻ on nitrogenase activity. It is also possible that the low activities of GS, directly or indirectly, are important in the sustenance of a particularly active nitrogenase in such symbiotic algae.

To localise NADP-dependent GDH activity cytochemically, sections of *P. canina* thalli and of *P. aphthosa* cephalodia were stained with blue tetrazolium (British Drug Houses, Poole, 0.25 mg ml⁻¹) in the presence of the GDH substrates NADP⁺ (1 mg ml⁻¹) and glutamate (20 mg ml⁻¹). With *P. canina*, activity was highest in the fungal hyphae closest to the blue-green algae, whereas in *P. aphthosa* cephalodia,

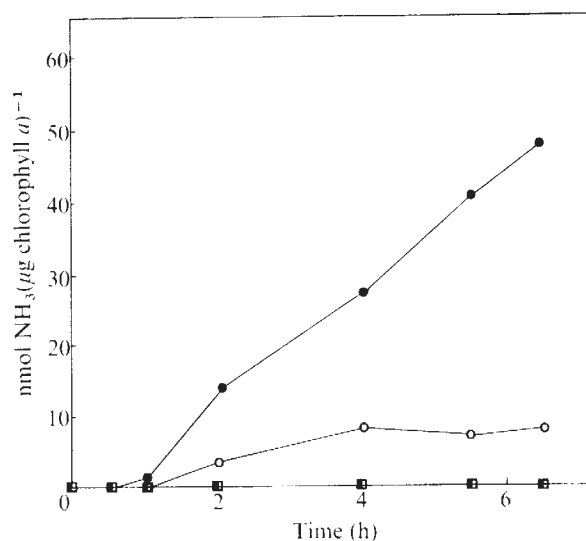


Fig. 1 Time course of production of extracellular NH₃ by 1.0-cm diameter disks of *Peltigera canina* incubated in air + digitonin (●), A/O₂/CO₂ (77.96:22.00:0.04, v/v) + digitonin (○), air (■), and A/O₂/CO₂ (■). In each experiment 30 disks were incubated with gentle shaking in 50 ml of N-free culture medium¹⁷ at 3,000 lx and 25 °C. NH₃ was determined according to Solorzano¹⁸.

Table 2 Activities of NH_3 -assimilating enzymes of *Peltigera aphthosa*, *Peltigera canina* and of the free-living *Nostoc* of *P. canina*

Lichen	Material	Enzyme activities (nmol per mg protein per min)			
		GS	GDH (NADP ⁺ -dependent)	ADH	GOT
<i>P. canina</i>	Intact thallus	0.8	201.7	3.8	41.0
	Symbiotic <i>Nostoc</i>	4.1	2.4	5.5	16.8
	Free-living <i>Nostoc</i>	72.4	ND	22.9	40.4
<i>P. aphthosa</i>	Intact thallus	25.7	90.2	—	58.4
	Excised cephalodia	4.5	401.1	—	20.2
	Thallus—cephalodia	17.5	34.8	—	47.0

Peltigera canina and *P. aphthosa* were collected in the field and maintained in the laboratory on N-free medium at 18 °C and 3,000 lx for 24 h before use. The symbiotic *Nostoc* was separated from the remainder of the *P. canina* thallus by the method of Millbank²⁰. The free-living phycobiont of *P. canina*, provided by Dr J. W. Millbank, was grown in N-free medium in continuous culture at 25 °C and 3,000 lx. Before enzyme assay the material was washed in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM β -mercaptoethanol and 5 mM ethylene diamine-tetraacetic acid. After homogenisation of the lichen material, the homogenates, and the free-living algae, were passed through a French pressure cell at 16,000 pounds per square inch. The extracts were then centrifuged at 35,000g for 20 min and the supernatant was passed through Sephadex G-25 to remove low molecular weight compounds then assayed for enzymic activity. The glutamine synthetase (EC 6.3.1.2) biosynthetic assay was that of Kingdon *et al.*²¹. Glutamate dehydrogenase (EC 1.4.1.2.) was estimated by following the oxidation of NADPH at 340 nm in a reaction mixture (3.0 ml final volume): 300 μ mol Tris-HCl buffer, pH 7.5; 0.3 μ mol NADPH; 10 μ mol 2-oxoglutarate; 400 μ mol NH_4Cl . The glutamate-aspartate-aminotransferase (EC 2.6.1.12) and alanine dehydrogenase (EC 1.4.1.1) assays were those of Rowell and Stewart²². NADP⁺-dependent GDH was also measured in the above samples using the assay described above with the substitution of NAD⁺ and NADH for NADP⁺ and NADPH. Activity was detected only in the intact thallus (3.8 nmol per mg protein per min) and in the excised cephalodia (4.6 nmol mg per protein per min) of *P. aphthosa*; ND, not detectable; —, not tested.

hyphae surrounding the algae stained heavily but the algae were unstained. The fungal hyphae did not stain in the absence of GDH substrates. Thus, if the N fixed by the symbiotic algae is excreted as NH_3 , as would be expected if the primary route of NH_3 assimilation by the algae is blocked, this NH_3 could be assimilated by the fungal GDH.

Finally, we investigated whether the symbiotic *Nostoc* of *P. canina* excreted NH_3 . First, disks of *P. canina* were incubated under $\text{A/O}_2/\text{CO}_2$ (77.96:22.00:0.04, v/v) or air in the presence and absence of 0.01% (w/v) digitonin, which affects fungal membrane permeability and depresses metabolite assimilation by the symbiotic fungus¹³. Figure 1 shows that during 6.5 h in N_2 , but not in its absence, NH_3 was liberated into the medium when digitonin was present. No NH_3 was excreted in the absence of digitonin, and digitonin did not affect nitrogenase activity during the experiment.

Next, *P. canina* disks were exposed to $^{15}\text{N}_2$ in the presence of digitonin and the labelling of the extracellular NH_3 and other nitrogenous products was noted. As Table 3 shows, at the end of the experiment the ^{15}N labelling of the extracellular NH_3 was higher than that of the lichen disks and of the organic extracellular N, 50% of the total ^{15}N fixed remained in the disks and, of the remaining ^{15}N incorporated, 50% was released extracellularly as organic N and 50% as extracellular NH_3 . Thus as with the symbiotic

algae of *Blasia pusilla*¹⁴ and *Azolla*¹⁵, much of the newly fixed N was excreted as NH_3 .

The above modifications of the symbiotic algae seem to depend on the eukaryotic partner(s) since, as in *Blasia pusilla*^{14,16}, when the blue-green alga is incubated free from its partners, it reverts to the morphology, physiology and biochemistry of typical free-living forms. Further work is necessary to show whether the eukaryotes produce specific effectors which regulate GS and possibly other NH_3 -assimilating enzymes in the cyanophyte, or whether the changes are due solely to internal regulation by the cyanophyte of its metabolism as a result of the presence of the eukaryotes.

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Table 3 Incorporation of ^{15}N from $^{15}\text{N}_2$ into disks, extracellular NH_3 and extracellular organic N of digitonin-treated *Peltigera canina*

Material	Total N present (mg)	^{15}N -labelling of material (atom % excess ^{15}N)	% of total fixed N in each sample
Disks	70	0.022	50
Extracellular organic N	3.9	0.205	25
Extracellular NH_3	1.1	0.727	25

350 1.0-cm diameter disks of *Peltigera canina* were washed in N-free culture medium¹⁷ for 6 h then incubated in $\text{A/O}_2/\text{CO}_2$ (77.96:22.00:0.04, v/v) in N-free medium for 12 h. The gas phase was then replaced by $\text{N}_2/\text{A/O}_2/\text{CO}_2$ (10:67.96:22.00:0.04, v/v), the ^{15}N -enrichment of the N_2 being 44 atom % ^{15}N . (All ^{15}N assays were carried out by mass spectrometry using an Associated Electrical Industries MS20 mass spectrometer.) Digitonin (0.01%, w/v) was also added at 0 time. After 21 h at 1,000 lx and 25 °C the total nitrogen and ^{15}N -labelling of the disks, extracellular NH_3 and extracellular organic nitrogen were determined²³. The ^{15}N -label of the gas phase did not change over the experimental period. The experiment was run in duplicate.

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Model for the regulation of nitrate assimilation

NITRATE assimilation is one of the two major biological processes by which inorganic nitrogen is converted to ammonia and thence to organic nitrogen. Photosynthetic organisms, with the possible exception of some blue-green algae and plants that have a symbiotic association with nitrogen-fixing bacteria, derive most of their nitrogen from nitrate¹. The rate-controlling and regulated step in the process of nitrate assimilation seems to be the conversion of nitrate to nitrite, catalysed by the enzyme nitrate reductase². There is a good correlation between the activity of this enzyme and the yield of grain protein in several cereal crops³⁻⁵. Nitrate arising from chemical fertilisers and industrial wastes is also a major factor contributing to the growth of algae and other microorganisms, leading to the eutrophication of lakes and streams. Thus, the control of nitrate assimilation can be important from the standpoint of both agricultural productivity and water resource management.

In this communication we propose a model (Fig. 1) for the regulation of nitrate assimilation in which CO₂ fixation and nitrate assimilation are coordinately controlled by the intracellular ratio of [O₂]/[CO₂]. This model accounts for the observed effects of O₂, CO₂ and light on nitrate assimilation and provides a rationale for the *in vivo* regulation of nitrate reductase by cyanide.

The nitrate reductase from *Chlorella* and several other

organisms can exist in either an active or in an inactive form (see ref. 6). *In vitro* experiments with purified nitrate reductase from *Chlorella vulgaris* showed that two components are required for the rapid and reversible inactivation of nitrate reductase⁷. The most potent combination of effectors is the physiological reductant NADH, and cyanide. Cyanide reacts stoichiometrically with the NADH-reduced enzyme to produce a stable enzyme-cyanide complex having a dissociation constant of about 10⁻¹⁰ M (ref. 8). These results indicated that very low concentrations of cyanide are sufficient to convert the active enzyme to an inactive form. The conversion of the inactive form to the active form by treatment with an oxidant is accompanied by the release of bound cyanide⁹. Nitrate reductase can be inactivated *in vivo* by brief incubation of the cells with ammonia⁹. The *in vivo* inactivated nitrate reductase isolated from such cells contains a stoichiometric amount of bound cyanide that is likewise released on conversion of the inactive nitrate reductase to the active form⁸. These results suggested that cyanide is a physiological regulator of nitrate reductase activity.

Pistorius *et al.*¹⁰ have shown that the *in vivo* inactivation of *Chlorella* nitrate reductase is stimulated by O₂ and inhibited by CO₂ and nitrate. Both the effect of O₂ and that of CO₂/nitrate are light dependent¹⁰. O₂ also inhibits photosynthetic CO₂ fixation¹¹. Ribulose 1,5-diphosphate (RuDP) carboxylase can function either as an oxygenase or as a carboxylase^{12,13}. Lorimer and Andrews¹⁴ proposed that in the reaction of RuDP with the enzyme, an enolate anion of RuDP is generated which would be susceptible to attack by either CO₂ or O₂. CO₂ fixation could therefore be directly regulated by the ratio of [O₂]/[CO₂]. As indicated in the

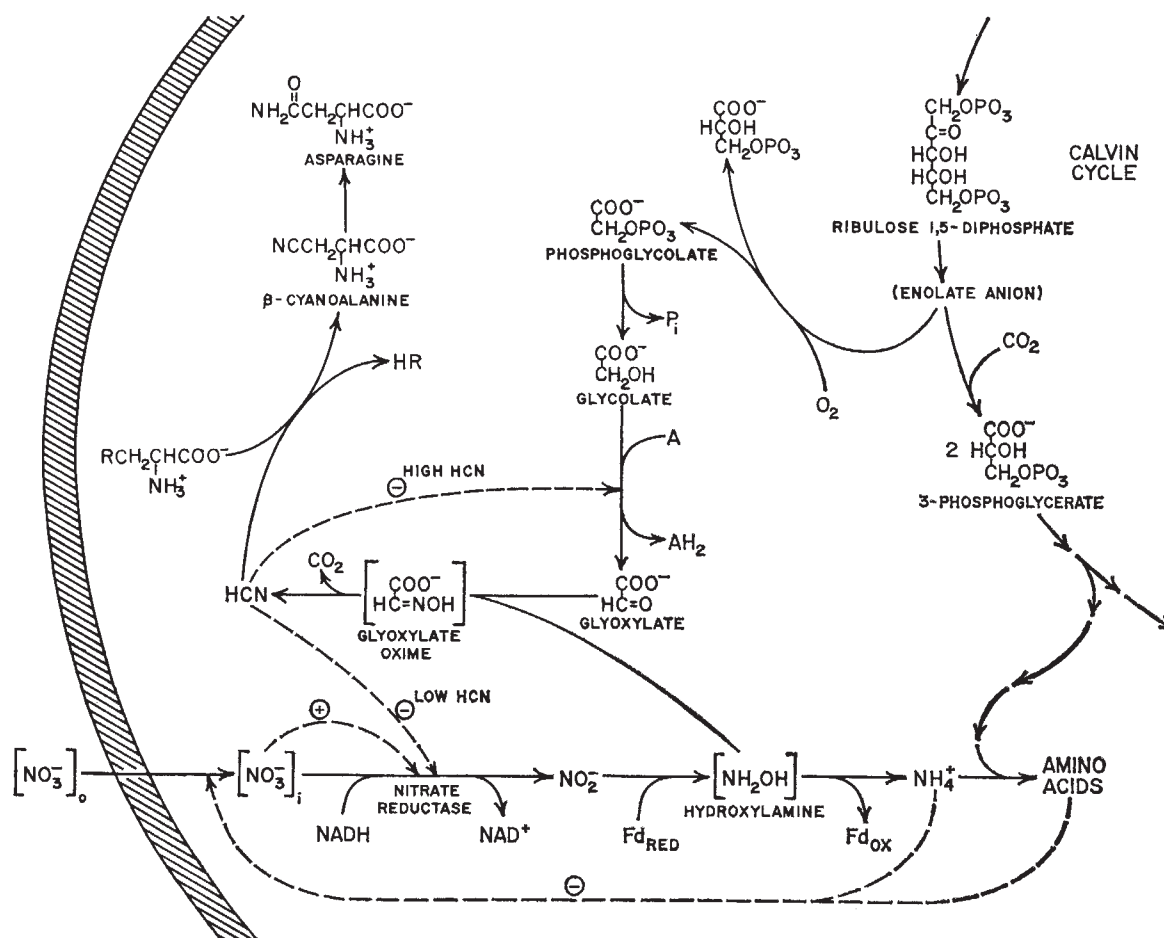


Fig. 1 Model for the regulation of nitrate assimilation. Solid lines represent metabolic conversions. Dashed lines represent inhibition (—) or activation (+) reactions.

model, phosphoglycolate, one of the products of the oxygenase reaction, can be converted to glycolate and thence to glyoxylate¹⁵. In green algae the oxidation of glycolate to glyoxylate is catalysed by a cyanide-sensitive glycolate dehydrogenase¹⁶. The physiological hydrogen acceptor for this reaction is not known.

Loussaert and Hageman have recently reported that hydroxylamine is a product of *in vitro* nitrite reduction in the presence of ammonia¹⁷. A central feature of our proposed model is that hydroxylamine reacts with glyoxylate which is then converted to cyanide and CO₂. This proposal is supported by experiments showing that sonicated extracts¹⁸ of *Chlorella vulgaris* catalyse the formation of cyanide from hydroxylamine and glyoxylate. These reactions were carried out at room temperature in sealed Conway diffusion dishes that contained 0.5 ml of 0.5 N NaOH in the centre well and 2.0 ml of reaction mixture in the outer well. The complete reaction mixture contained 40 mM HEPES NaOH (pH 7.5), 5 mM sodium glyoxylate, 5 mM hydroxylamine and 1 mM MnCl₂. The reaction was started by the addition of cell extract and was terminated at various time intervals by the addition of 0.2 ml of 50% trichloroacetic acid to the reaction mixture. The dishes were immediately resealed and incubated for an additional 30 min at room temperature to allow complete diffusion of the HCN to the alkali trap. The amount of cyanide in the centre well was then measured by the method of Guilbault and Kramer¹⁹. The rate of cyanide formation was about 1 nmol min⁻¹ mg⁻¹. The activity of nitrate reductase (after activation⁷) in these extracts was about 150 nmol NO₃ reduced min⁻¹ mg⁻¹. No significant formation of cyanide was observed in the following controls: (1) absence of hydroxylamine or glyoxylate, (2) with heat-inactivated extracts, or (3) ammonia substituted for hydroxylamine in the complete reaction mixture. The rate of cyanide formation was essentially constant over a 60-min incubation period, was directly proportional to the amount of extract added to the reaction mixture and was stimulated by Mn²⁺. This stimulation by Mn²⁺ is consistent with the observation that the formation of inactive nitrate reductase, *in vivo*, is impaired in Mn²⁺-deficient cells¹⁰. Like nitrate reductase, the enzyme which catalyses the formation of cyanide from hydroxylamine and glyoxylate is found in the supernatant after centrifugation at 100,000g for 1 h.

Only very low levels of cyanide (~10⁻⁹ M) would suffice for the reversible inactivation of nitrate reductase⁸. The concentration of HCN required for the inactivation of nitrate reductase is several orders of magnitude smaller than that which would inhibit respiration^{8,20}. The formation of high levels of cyanide could be prevented by the feedback inhibition of glycolate dehydrogenase at higher concentrations of cyanide (~10⁻⁶ M) (ref. 16). This aspect of the model is also supported by the observation that *Chlorella* cells excrete glycolate at high light intensity, high O₂ tension and low CO₂ tension²¹, the same conditions that cause maximum reversible inactivation of nitrate reductase¹⁰. Added HCN mimics these effects of high O₂ tension²², suggesting that the cyanide formed internally when cells are illuminated in the presence of high [O₂] causes an accumulation of glycolate.

Excess cyanide could be removed by a reaction sequence in which cyanide is converted to the β -carboxamide of asparagine. Exogenous cyanide is converted to the β -carboxamide group by seedlings of a variety of plants²³. Fowden and Bell²⁴ have also shown that seedlings and *Chlorella* convert H¹⁴CN to β -cyanoalanine which in turn is converted to asparagine or to the peptide γ -glutamyl- β -cyanoalanine. The feedback regulation of cyanide formation and its removal by the above 'scavenger' pathway would prevent the accumulation of cyanide and the inhibition of other vital metabolic processes such as respiration.

An additional feature of the model is the inhibition of

nitrate uptake by ammonia and/or amino acids. A nitrate-uptake system that is independent of nitrate reductase and that is inhibited by ammonia or amino acids has been demonstrated in cultured tobacco cells²⁵ and in *Neurospora crassa*²⁶.

A rapid *in vivo* activation of nitrate reductase, independent of *de novo* enzyme synthesis has heretofore not been demonstrated unequivocally. Nitrate, in conditions of high light intensity, high [CO₂] and absence of ammonia causes an apparent *in vivo* conversion of the inactive form of nitrate reductase to the active form^{9,10}. Cycloheximide partially inhibits this *in vivo* 'reactivation' of nitrate reductase by nitrate¹⁰, suggesting that the reactivation may be at least partially dependent on *de novo* protein synthesis. Inhibitors of protein synthesis may, however, affect the intracellular concentrations of amino acids thereby inhibiting the nitrate-uptake system^{25,26}. This might explain the apparent inhibition of the *in vivo* activation of nitrate reductase by cycloheximide.

It has been demonstrated that when tungsten is substituted for molybdenum in the growth medium, a tungsten

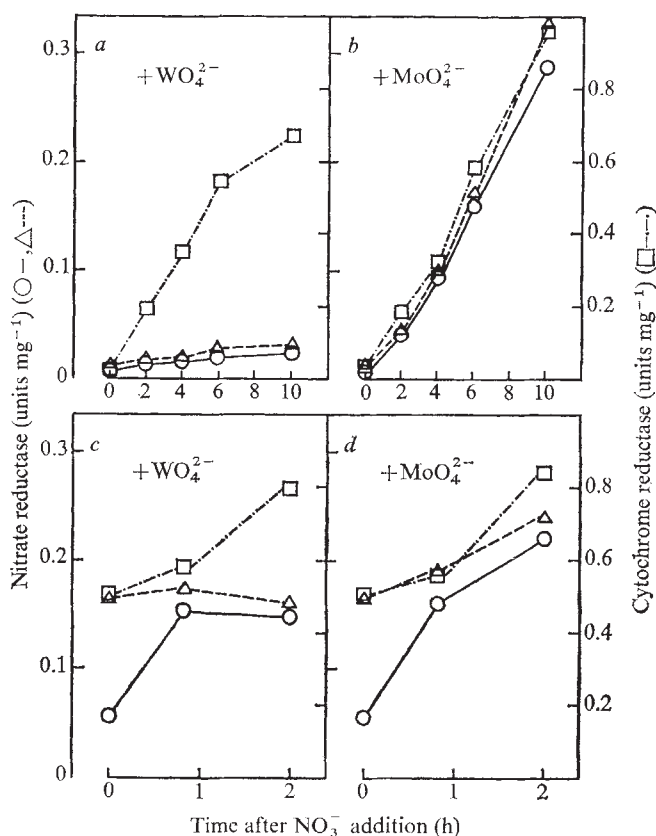


Fig. 2 Nitrate-induced synthesis and *in vivo* activation of nitrate reductase by nitrate. For *a* and *b*, *Chlorella vulgaris* cells were cultured in an ammonium growth medium²⁹ for 4 d and then were collected and transferred to growth medium containing 10 mM KNO₃ and either 10 μ M Na₂WO₄ (*a*) or 10 μ M Na₂MoO₄ (*b*). Aliquots of these cell cultures were withdrawn at the indicated intervals, harvested and disrupted by sonication in 10 mM HEPES (pH 7.5) and 2 mM KNO₃. The sonicated extract was centrifuged for 10 min at 25,000g (4 °C). The supernatant was assayed for NADH-cytochrome *c* reductase activity ($\square$ — $\square$) and NADH-nitrate reductase activity before ($\circ$ — $\circ$) and after ($\triangle$ — $\triangle$) a 2 min incubation with 2 mM potassium ferricyanide to convert the inactive form of nitrate reductase to the active form⁷. Activity is expressed as units (μ mol min⁻¹) per mg of protein. The conditions were the same for *c* and *d* except that nitrate-grown cells were pre-incubated in the ammonium growth medium for 2 h before transfer to growth medium containing 10 mM KNO₃ and either 10 μ M Na₂WO₄ (*c*) or 10 μ M Na₂MoO₄ (*d*). This short pre-incubation with ammonium causes an *in vivo* inactivation of nitrate reductase^{8,30} in contrast to the disappearance of nitrate reductase and associated cytochrome *c* reductase as occurs on long term cultivation in ammonium-containing medium (*a* and *b*).

analogue of nitrate reductase is formed which has NADH-dehydrogenase (cytochrome *c* reductase) but no nitrate-reducing activity^{27,28}. Thus, in the presence of tungstate and absence of molybdate, any new nitrate reductase formed would be expected to have cytochrome reductase activity but no nitrate-reducing activity. By contrast, any increase in nitrate-reducing activity due to the *in vivo* activation of inactive nitrate reductase should be unaffected by tungstate. Therefore, with the use of tungstate, it should be possible to distinguish unequivocally between *de novo* enzyme synthesis and *in vivo* activation of nitrate reductase.

When *Chlorella* cells are exposed to ammonium for long periods (>24 h), the total amount of nitrate reductase, including the associated cytochrome *c* reductase activity, falls to <5% of the original level²⁹. When such cells were transferred to a nitrate medium containing tungstate (Fig. 2a), there was a normal development of cytochrome *c* reductase activity (see Fig. 2b) but no significant development of nitrate reducing activity. Thus, enzyme synthesised in the presence of tungstate has no nitrate-reducing activity. For the experiment shown in Fig. 2c and d, *Chlorella* cells were exposed to ammonium for 2 h. This short treatment with ammonium causes an *in vivo* inactivation of nitrate reductase^{8,30}, that is there is no significant change in cytochrome *c* reductase and total nitrate reductase activities but there is a decrease in % active nitrate reductase. When these cells were then transferred to a nitrate medium containing tungstate (Fig 2c), there was a normal development of nitrate-reducing activity (measured before activation) over the first 45 min (see Fig. 2d). Further development of nitrate-reducing activity, which presumably reflects *de novo* enzyme synthesis, was inhibited by tungstate. These results indicate that the *in vivo* activation of nitrate reductase is independent of *de novo* enzyme synthesis.

Although not indicated in the proposed model, light would have an important role in this regulatory mechanism since ATP and NADPH derived from the light reactions of photosynthesis would be required for the generation of RuDP, the substrate for the reactions catalysed by RuDP carboxylase/oxygenase. As would be predicted from our model, the *in vivo* inactivation and activation of nitrate reductase are both stimulated by light¹⁰. Also of possible relevance to the proposed model are recent results of Lips and associates³¹⁻³³ which suggest that enzymes of CO₂ fixation (RuDP carboxylase), photorespiration (glycolate oxidase) and nitrate assimilation (nitrate reductase) are associated with microbody-like organelles which attach themselves to chloroplasts during illumination.

Since CO₂ fixation by the Calvin cycle is the ultimate source of carbon skeletons for the fixation of ammonia produced by assimilatory nitrate reduction, little or no fixation of ammonia could occur in the absence of CO₂. It is reasonable, therefore, that these two processes be coordinately regulated. When the rate of CO₂ fixation is low, the accumulation of potentially toxic intermediates of nitrate assimilation such as nitrite, hydroxylamine, and ammonia would be prevented. When the rate of CO₂ fixation is high, the formation of sufficient ammonia for the synthesis of organic compounds would be ensured. We propose that this coordinate regulation of CO₂ fixation and nitrate assimilation can be accomplished by the reactions outlined in our model.

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Membrane localised gibberellins A₉ and A₄ in wheat chloroplasts

GIBBERELLINS help to regulate plant growth and development, but their mechanism of action is still not clear¹. Attempts to correlate the levels of solvent-extractable gibberellins in plants with specific developmental events have shed little light on these problems. Turnover rate is one variable of possible physiological importance not accounted for when steady state levels of extractable hormone are measured. Another relevant factor is the way in which the hormones are sequestered or compartmented within the cell; this may reflect both how the hormone acts and is controlled and how it can be extracted. Prompted by recent evidence that gibberellins are released from wheat² and barley³ etioplasts *in vitro* by red light, we investigated whether gibberellins are associated with membranes in wheat chloroplasts. We found that gibberellins (principally GA₉ and GA₄) can be extracted from chloroplast membrane preparations using novel extraction procedures. Quantities found were much greater than the amounts of gibberellins previously extracted from the vegetative tissues of any plant species.

The non-ionic detergent Triton X100 (ref. 4) was used to disperse membranes in chloroplast preparations obtained from the first leaves of 8-d-old seedlings of *Triticum aestivum* variety Kolibri raised under long photoperiods in a heated greenhouse. Leaves in 10-g lots were ground in a mortar and pestle lined with a 50-μm mesh nylon cloth for 2 min at 2 °C in 100 ml of a modified Honda⁵ medium at pH 7.2 (9% sucrose; 25 mM potassium-Tricine buffer; 8 mM 2-mercaptoethanol; 5 mM magnesium acetate; Dextran-15; (50 mg ml⁻¹); Ficoll (25 mg ml⁻¹); bovine serum albumin (1 mg ml⁻¹). Material not retained by the mesh was centrifuged at 1,000g for 10 min and the supernatant was discarded. Figure 1a and b shows that about 1,000 times more gibberellin activity of low polarity (*R_f* 0.5-0.7) was present in Triton-extracted than in methanol-extracted chloroplast preparations. A more polar fraction (*R_f* 0.1-0.2) was present at relatively low and approximately equal levels in the two extracts. Figure 1c shows that only slightly more

gibberellin activity was found when the pellet was disrupted by sonication before methanol extraction than when the intact preparation was extracted with methanol directly. The gibberellin activity detected in the methanol extracts was comparable with the activity found when an equivalent weight of leaves was extracted directly with 80% methanol and subjected to the same purification procedure.

Most of the gibberellins so far identified in higher plants have been obtained from immature seeds, because the very low levels hitherto found in vegetative tissues have made identification extremely difficult. The levels of the less polar gibberellins extracted from crude chloroplast preparations with Triton are similar to the gibberellin levels found in methanolic extracts of immature seeds. Hence we were able to identify the gibberellins extracted by Triton without recourse to complex purification procedures. After the usual initial purification steps (Fig. 1), the bulk of gibberellin activity could be resolved into two major, relatively non-polar fractions using silicic acid partition column chromatography⁸. The less polar of these fractions was methylated with ethereal diazomethane and subjected to combined gas chromatography-mass spectrometry (GCMS). Mass spectra were recorded at 3 s per mass decade using an AEI MS30 mass spectrometer with a DS50 data system. Ions of mass 330 (M^+) 298 270 and 271, characteristic of methyl GA_3 , were detected at the retention time of authentic methyl GA_3 using the DS50 cross scan facility. GA_3 was identified unequivocally when an exceptionally clean mass spectrum was obtained from a Triton extract subjected to an additional purification step in which the methylated extract underwent Gelman instant thin-layer chromatography⁹ (ITLC) in benzene:petroleum ether (boiling point 60–80 °C) (3 : 1 v/v). The zone corresponding in R_f with authentic methyl GA_3 was eluted with ethyl acetate before GCMS.

After the same procedures but with petroleum ether :

diethyl ether (7 : 3, v/v) as the ITLC solvent, the more polar fraction from the partition columns was trimethylsilylated after ITLC and, on GCMS, yielded a mass spectrum identical with that of authentic methyl trimethylsilyl GA_4 . These identifications will be fully documented elsewhere. None of the gibberellins in wheat plants have been identified before.

We were able routinely to use gas chromatography-single ion current monitoring (GC-SICM) to determine the gibberellin content of partially purified extracts as described in the legend to Table 1. Determined thus, the levels of GA_3 and GA_4 in Triton and methanol extracts of crude chloroplast preparations were similar to the levels measured by bioassay.

To check whether the Triton extractable gibberellins were associated primarily with the chloroplasts, 1,000 g crude chloroplast pellets obtained in the usual way were resuspended in 20 ml of extraction medium and eluted with more medium through a 38 × 3 cm column of loosely packed Sephadex G-50⁸. The first green band to elute from the column (1.5 V_0) consisted almost entirely of intact chloroplasts as seen with phase contrast and electron microscopy. Chloroplasts prepared thus contain per unit chlorophyll the same levels of methanol and Triton-extractable GA_3 as the crude 1,000g pellet. That the bulk of the gibberellins are released only by detergent treatment, and not by sonication, suggests an intimate association with the chloroplast membranes. Further evidence in support of this hypothesis was obtained as follows. 1,000g pellets were either resuspended in the usual medium and then sonicated or resuspended in the usual medium without sucrose to lyse the chloroplasts osmotically. The disrupted chloroplasts were centrifuged at 20,000g for 20 min and the pelleted membranes were extracted with Triton or methanol, treated as usual. The gibberellins extracted with Triton were found by GC-SCIM and by bioassay to be in

Fig. 1 Gibberellin activity in extracts of the crude 1,000g pellet derived from 10 g fresh weight leaf samples. *a*, Pellet resuspended in 50 ml of 2% Triton X100 buffered at pH 8.0 in 25 mM potassium Tricine. *b*, Pellet resuspended in 100 ml of methanol : water at pH 8.0 (4 : 1, v/v). *c*, Pellet resuspended in 20 ml of grinding medium, disrupted by three 15-s bursts from an MSE 150 W sonicator (nominal frequency 20 kHz), diluted with 80 ml methanol and, after 1 h, centrifuged at 40,000g for 1 h and the supernatant taken for further processing. The 40,000g pellet from (*c*) resuspended in 50 ml of 2% Triton X100 at pH 8.0. All extracts were subjected to a standard initial purification procedure. Methanol was removed under reduced pressure on a rotary evaporator at 35 °C. Extracts were diluted to 200 ml with distilled water, adjusted to pH 8.0 and passed through a 2 × 10 cm column of Dowex IX1-100 formate; the column was washed with a further 200 ml water at pH 8.0. Gibberellins were eluted from the resin with 400 ml of ethanol : 1.0 M formic acid (4 : 1 v/v). The eluate was reduced to a small volume of aqueous solution on a rotary evaporator, diluted with 100 ml of 0.1 M Na_2HPO_4 , adjusted to pH 8.0 and stirred for 1 h with 20 g of insoluble polyvinyl pyrrolidone (PVP)⁶. After filtration the extract was adjusted to pH 3.0 and partitioned three times against half its volume of ethyl acetate. Bulked ethyl acetate extracts were dried with anhydrous magnesium sulphate and the solvent was removed on a rotary evaporator. For bioassay, purified extracts dissolved in methanol were applied to 10-cm wide sheets of Whatman No. 1 chromatography paper. Chromatograms were developed in *iso*-propanol : 0.880 : ammonia : water (10 : 1 : 1, v/v/v), cut into strips corresponding to 0.1 - R_f units and the strips bioassayed directly using the lettuce hypocotyl test⁷.

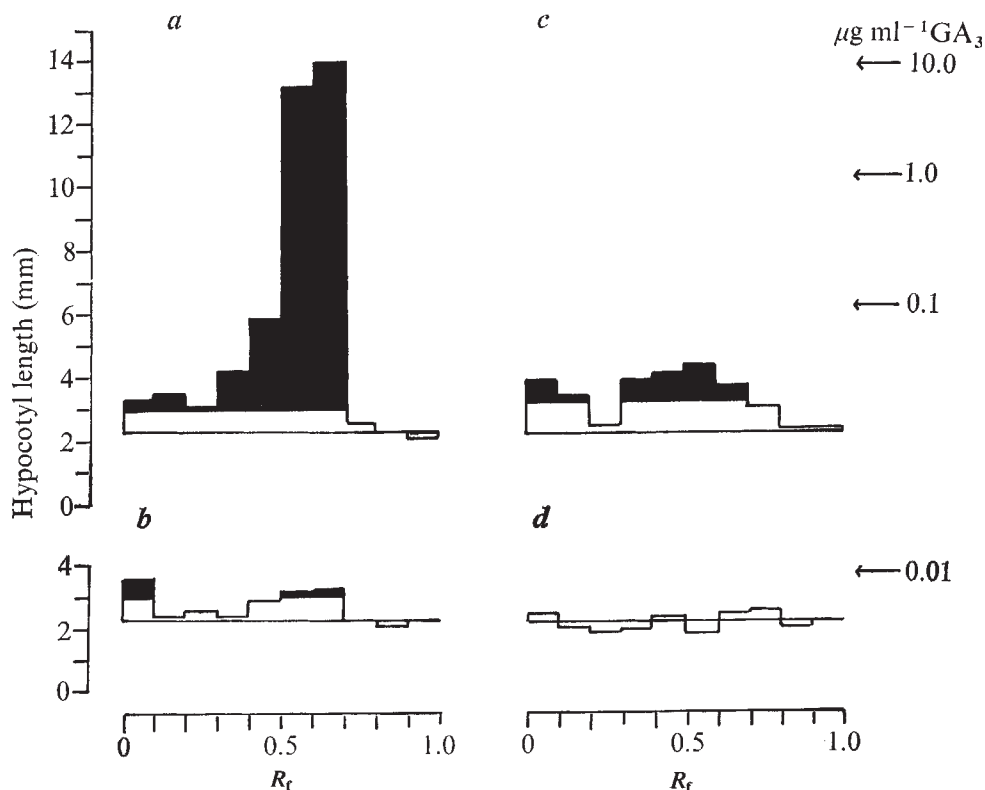


Table 1 Yields of GA₉ and GA₄ from chloroplast membrane preparations

Extraction procedure	Assay method			
	GC-SICM		Bioassay ($\mu\text{g GA}_9$ equivalents)	
	GA ₉	GA ₄	GA ₉	GA ₄
Triton X100 extracts ($\mu\text{g mg}^{-1}$ chlorophyll)	2.9	0.9	2.1	0.5
($\mu\text{g g}^{-1}$ fresh weight leaves (adjusted for membrane recoveries))	7.7	2.4	5.9	1.4
80% Methanol extracts ($\mu\text{g mg}^{-1}$ chlorophyll)	0.005	0.001	0.004	0.001
($\mu\text{g g}^{-1}$ fresh weight leaves (adjusted for membrane recoveries))	0.013	0.004	0.01	0.003

Crude chloroplast pellets from 10 g fresh weight of leaves were resuspended in 20 ml extraction medium and sonicated as before. Sonicated preparations were centrifuged at 20,000g for 20 min and the pelleted material was extracted with Triton or aqueous methanol. The extracts were partially purified (Fig. 1) and for GC-SICM the ethyl acetate extracts were methylated with ethereal diazomethane. The methylated extracts were subjected to ITLC using benzene-petroleum ether (3 : 1, v/v) as solvent for methyl GA₉ or petroleum ether-diethyl ether (7 : 3, v/v) as solvent for methyl GA₄; zones corresponding in R_f to those of authentic methyl GA₉ or methyl GA₄ were eluted with ethyl acetate. The ethyl acetate was removed on a rotary evaporator and the extract was redissolved in 2 ml of acetone. Methyl-GA₉ or methyl-GA₄ content was measured by injecting 1 μl of the acetone extract on to a glass column (182 cm $\times$ 0.4 mm inner diameter) packed with 2% OV-1 coated on Gas Chrom Q (100–120 mesh) fitted to a Pye 104 gas chromatograph coupled through a silicone rubber membrane separator to an AEI MS 30 mass spectrometer. Samples were chromatographed isothermally at 228 °C with 40 ml min⁻¹ helium carrier gas. The MS 30 was set to monitor current at an m/e value of 298 for GA₉ or 224 for GA₄ with 1,000 resolving power using authentic methyl GA₉ or methyl GA₄ as reference compounds. High frequency noise was eliminated by a filter circuit adjusted to give the minimum peak distortion and the selected ion current was recorded with a Servoscribe 1s pen recorder. The total ion current was recorded on a second Servoscribe. Authentic GA₉ added to 50 ml 2% Triton X100 could be recovered after these procedures in yields of > 90%. For bioassay, acidic ethyl acetate extracts were line loaded on to 10 $\times$ 20-cm thin-layer chromatography plates spread with 250 μm silica gel. Plates were developed in ethyl acetate-chloroform-acetic acid (15 : 10 : 1 v/v) and dried overnight over a fan. The silica gel was removed in bands corresponding to 0.1 R_f values and bioassayed directly using the lettuce hypocotyl test⁷. Authentic GA₉ moved in the solvent system used to R_f 0.85–0.9 and GA₄ to 0.65–0.7. Chlorophyll was determined according to Arnon¹⁰.

the membrane pellets (Table 1), which per unit of chlorophyll contained similar amounts of gibberellin as intact chloroplasts. Extracted separately with methanol, the membrane pellet and supernatant yielded approximately equal amounts of gibberellin; combined pellet-supernatant gibberellin content was similar to that of intact chloroplast preparations extracted by methanol.

Circumstantial evidence suggests that the Triton-extractable gibberellins are localised within the chloroplast membranes in a specific manner, rather than simply partitioned into the membrane lipids. The gibberellins are not extracted by chloroform:methanol mixtures which are reported to delipidate membranes completely¹¹. And they are not extracted from sonicated chloroplast preparations by 8 M urea or by 1 mM EDTA, which would be expected respectively to release components linked to the periphery of the membrane by hydrogen bonds or by metallic cations¹². One possibility is that the Triton-extractable gibberellins are intimately associated with non-polar proteins or other molecules located within rather than on the periphery of the membranes. This might explain not only why the Triton-extractable gibberellins are not extracted with methanol but also why methanol renders them not extractable with Triton. The detergent failed, for example, to extract gibberellins from the insoluble residue which remained when the chloroplasts were sonicated and extracted for 1 h with 80% methanol and then centrifuged at 40,000g for 1 h (Fig. 1d). Furthermore, the amounts of gibberellin extracted by methanol were not increased when Triton was added to unfractionated methanolic extracts or when methanol and Triton were added together. Possibly, methanolic extraction denatures or alters the proteins or other molecules with which the Triton-extractable gibberellins are intimately associated, in such a way that the gibberellins are rendered not extractable even by Triton.

Clearly, there seem to be at least two fractions of extractable gibberellins compartmented within the chloroplast membranes—one released from the membranes by methanol and another released by Triton. Whether these fractions belong to the same physiological pool, part of which is not efficiently extracted by organic solvents, or whether the two fractions are physiologically distinct needs to be determined.

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Removal of 5-bromo-2-deoxyuridine incorporated in DNA of regenerating rat liver

THERE are many systems available for the study of *in vitro* DNA repair, including autoradiography¹, inhibition of *de novo* DNA synthesis by hydroxyurea (HU)² and use of radioactive³ or cold 5-bromo-2-deoxyuridine (BUdR) as substrates, followed by density gradient separation⁴ or photoirradiation⁵ at 313 nm. There are a few methods available for *in vivo* studies of DNA repair⁶ based on indirect techniques involving the use of DNA alkaline sucrose gradient centrifugation, or on the use of HU and gel filtration in tissues with low mitotic rhythm (unpublished work). Use of BUdR in DNA labelling does not give satisfactory results *in vivo*, and autoradiography is impractical. We have examined the hypothesis that BUdR, once incorporated in newly-synthesised DNA, can be recognised as a steric encumbrance and so be removed by nuclear DNA repairing enzymes. This removal does occur, and forms the basis of a reliable method for measuring DNA repair in tissues with high DNA-synthetic activity.

Female Wistar rats (in groups of five) weighing 130–150 g underwent partial hepatectomy⁷; 23 h later, that is at the peak of the S phase, a mixture of ³H-thymidine (TdR) and ¹⁴C-BUdR (18.5 Ci mmol⁻¹ and 60 mCi mmol⁻¹, respectively),

Table 1 Removal of ^{14}C -BUdR incorporated in DNA of regenerating rat liver

First experiment	0*	1	2	3	4	8	15
$^3\text{H}/^{14}\text{C}$ ratio	6.52 ± 1.52	6.72 ± 1.41	—	6.30 ± 2.40	—	$15.35 \pm 2.64^\ddagger$	$17.88 \pm 1.18^\ddagger$
^3H -TdR-DNA labelling (d.p.m. mg^{-1})	$12,000 \pm 850$	$10,500 \pm 646$	—	$4,805 \pm 573$	—	$3,889 \pm 466$	$3,384 \pm 397$
^{14}C -BUdR-DNA labelling (d.p.m. mg^{-1})	$1,840 \pm 221$	$1,563 \pm 188$	—	763 ± 91	—	253 ± 30	186 ± 31
Second experiment							
$^3\text{H}/^{14}\text{C}$ ratio	7.57 ± 0.55	9.30 ± 0.90	5.27 ± 0.93	—	$13.94 \pm 1.91^\ddagger$	$21.89 \pm 1.78^\ddagger$	$25.65 \pm 2.08^\ddagger$
^3H -TdR-DNA labelling (d.p.m. mg^{-1})	$12,596 \pm 1,512$	$10,502 \pm 1,260$	$5,600 \pm 661$	—	$4,603 \pm 549$	$4,082 \pm 460$	$3,499 \pm 420$
^{14}C -BUdR-DNA labelling (d.p.m. mg^{-1})	$1,664 \pm 140$	$1,129 \pm 136$	$1,063 \pm 128$	—	330 ± 40	186 ± 22	136 ± 20

*Zero time values were obtained from a group of rats killed 1 h after i.p. injection of $9.1 \mu\text{Ci } ^3\text{H}$ -TdR together with $1.3 \mu\text{Ci } ^{14}\text{C}$ -BUdR ($^3\text{H}/^{14}\text{C}$ ratio = 7) 23 h post-hepatectomy in female Wistar rats (130–150 g) in groups of five. Animals were killed at various times and radioactivity was counted on duplicated 1.5-mg DNA samples. Data are reported as mean $\pm$ s.e.m.

$^\ddagger 0.05 > P > 0.01$.

$^\ddagger P < 0.01$.

(Radiochemical Centre, Amersham) was injected intraperitoneally in the ratio $^3\text{H}/^{14}\text{C}=7$ ($9.1 \mu\text{Ci}$ TdR plus $1.3 \mu\text{Ci}$ BUdR). In the first experiment rats were killed 1 h and 1, 3, 8 and 15 d after injection, while in a second experiment rats were killed 1 h and 1, 2, 4, 8 and 15 d after treatment. The livers were removed and processed separately. DNA was extracted, and purified from the isolated nuclei using a phenol method⁸ and radioactivity was determined in 0.4-ml samples of supernatant made 0.2 N HClO_4 after centrifugation. Two samples (about 1.5 mg) of DNA extracted from each of the livers were dissolved in distilled water, acidified, filtered on Whatman GF/C glass fibre dishes, washed with ethanol and then ether and dried. Finally, 3 ml methylcellulosolve and 10 ml scintillation liquid (0.4% PPO and 0.005% POPOP in toluene) were added to each vial and the double labelling (^3H and ^{14}C) was counted in a previously calibrated Inter technique SL 32 spectrometer, provided with external (^{226}Ra) standardisation.

Table 1 shows removal of ^{14}C -BUdR from DNA in regenerating rat livers; results are expressed both as the mean of the ratios $^3\text{H}/^{14}\text{C}$ and as the mean of DNA specific activity for each isotope $\pm$ s.e.m. Figure 1 shows the variation in time of the DNA specific activity for ^3H and ^{14}C (multiplied by 7 to be normalised to ^3H) from the second

experiment. The finding of a ratio $^3\text{H}/^{14}\text{C}$ in DNA equal to that of injected TdR/BUdR is casual and due to preferential uptake of TdR, diluted in the cell nucleotide pool. In Fig. 2, liver growth is plotted against time, and the quantity of DNA is expressed both as mg and as mg g^{-1} of tissue. CsCl sedimentations of DNA samples confirmed that ^3H and ^{14}C labels were associated with the DNA peaks (1.705 density) in the previously reported ratios.

BUdR removal from DNA began 4 d after initial treatment and stopped during the eighth day, that is, within a period in which DNA synthesis is completed and liver mass and DNA quantity per unit of weight have practically returned to normal. We are not aware of any reports of BUdR removal *in vivo* by a purely physicochemical mechanism and so we consider the observed BUdR removal as an indication of DNA repair. An alternative explanation of the data might be that cells were killed following incorporation of labelled BUdR and TdR, and that released ^3H -TdR is preferentially incorporated into DNA. This is unlikely, however, because both BUdR (1.7×10^{-7} M) and TdR (3.8×10^{-9} M) were administered in doses well below toxic levels (10,000-fold and 1,000-fold lower, respectively)^{9,10}. The ^3H -TdR dose used was 100-fold lower than the mutagenic dose in mouse¹¹, and the dose given per g body weight

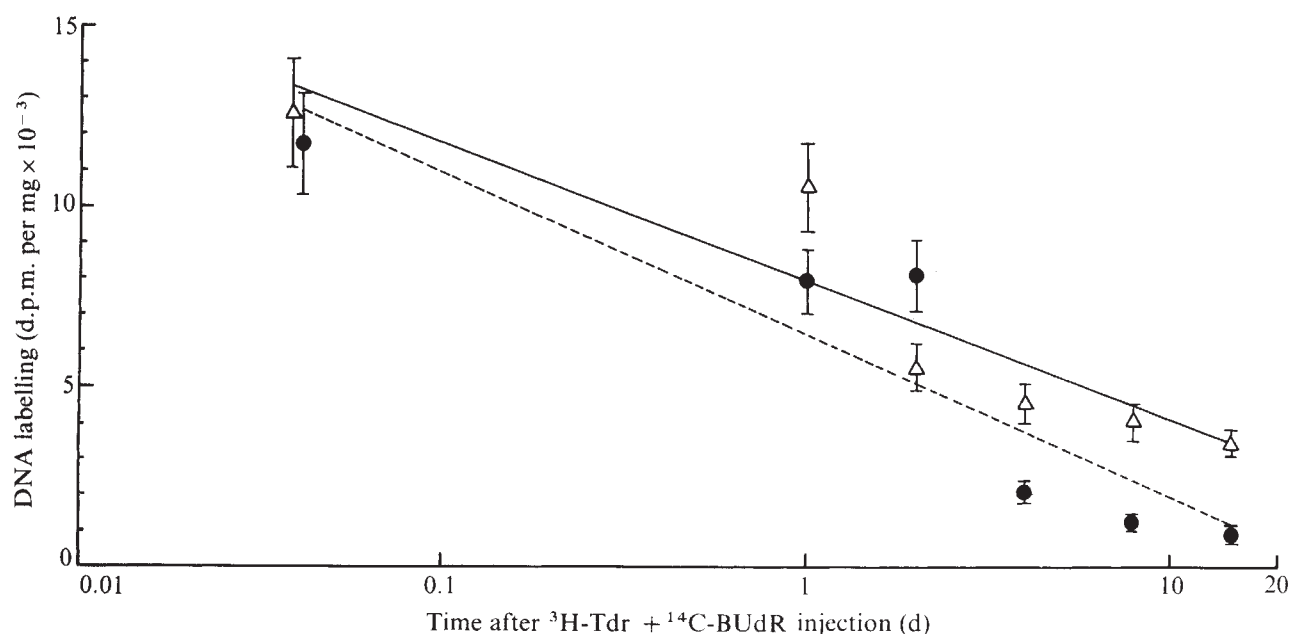


Fig. 1 DNA labelling time course after injection of $9.1 \mu\text{Ci } ^3\text{H}$ -TdR and $1.3 \mu\text{Ci } ^{14}\text{C}$ -BUdR (second experiment) to rats 23 h after hepatectomy (time 0). Data are the mean of duplicate estimations performed on five animals $\pm$ s.e.m. ^3H regression line: $y = 7851 - 3885.26x$ ($t = 28.83$). ^{14}C (normalised to ^3H) regression line: $y = 6461 - 4498.79x$ ($t = 38.34$); in both $x =$ time log. Slopes were significantly different at $P < 0.05$, when regression lines were compared.

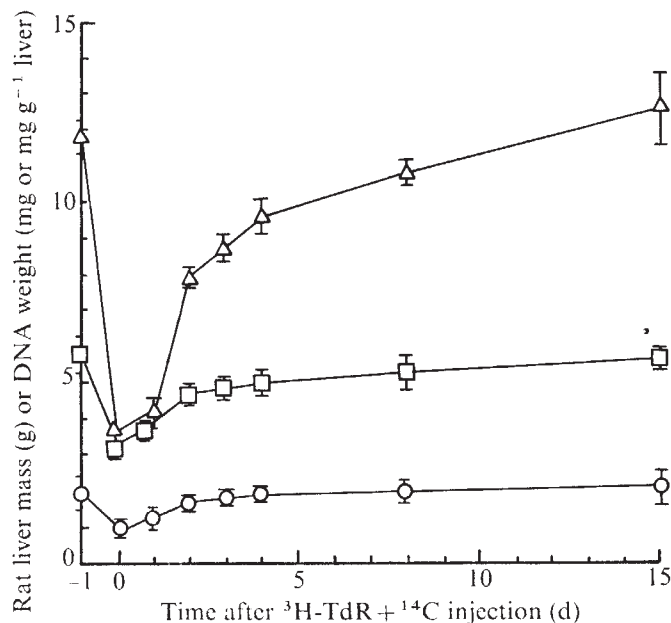


Fig. 2 Time course of rat liver mass as g (□) and DNA weight, both as mg (Δ) and as mg g⁻¹ tissue (○) after partial hepatectomy. Animals were injected with ³H-TdR/¹⁴C-BUDr as in Fig. 1, at 23 h after hepatectomy (time 0). Data are the mean five values ± s.e.m.

was sixfold lower than a dose which affects neither rat liver regeneration (as regards liver mass) nor mitotic rhythm and figures¹², in identical conditions. The dose of ³H-TdR we used *in vivo* does not influence cell viability when added to cell culture, an *in vitro* system where physiological removal of radioactive tracer is impossible¹⁰. On the basis of radioactivity incorporated into DNA, and assuming that there was 10 pg of DNA per cell, there were only 1.6 cell disintegrations within the first 8 d, and even fewer when it is considered that cell division occurs. Variations in ³H/¹⁴C ratio occurred when mitotic index was again low. Moreover BUDr is similarly removed in rat spleen cell cultures (our unpublished work) in the presence of excess cold TdR.

It is therefore possible that, when BUDr is inserted into DNA, there is filament breakage similar to that which occurs rapidly *in vitro* after photoirradiation, and that enzymatic repair can follow¹³. BUDr-induced breakage seems to take place more slowly than the radiation-induced process. Removal and the repair processes could follow the molecular mechanism involved in repairing γ-ray-induced damage⁵.

The method we describe can be used both to compare repair activity as a function of age in organs with high DNA synthesis and to study the relationship between carcinogenesis and DNA repair in systems, such as regenerating liver, for which data are available on tumour induction by single administrations of *N*-methyl-*N*-nitrosourea¹⁴, dimethylbenz(a)anthracene¹⁵, dimethylnitrosamine¹⁶ and urethane¹⁷.

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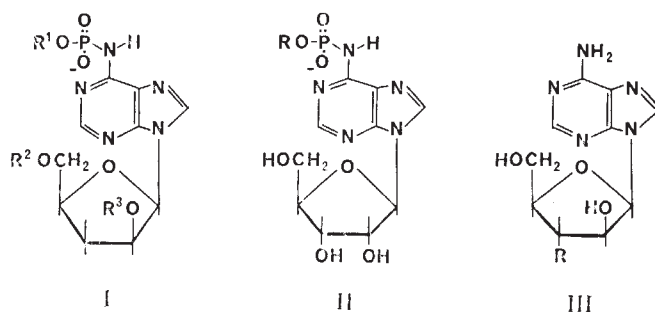
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Agrocin 84 is a 6-*N*-phosphoramidate of an adenine nucleotide analogue

We wish to draw attention to the natural occurrence of an extraordinarily selective antibiotic, agrocin 84, which is a 'fraudulent' member (structure I) of the hitherto unreported 6-*N*-phosphoramidate series of adenine nucleotides (structure II), of which 6-*N*-AMP (II, R=H) is the first member.



Kerr and Htay¹ have shown that the biological control of crown gall, achieved by the inoculation of susceptible plants with the non-pathogenic strain 84 of *Agrobacterium radiobacter*, is due to the production of a bacteriocin by the controlling organism. This bacteriocin, now known as agrocin 84, selectively inhibits the growth of converted pathogenic strains of *A. radiobacter*, while the growth of most non-pathogenic strains is unaffected²; this property is utilised in the bioassay, which was originally developed by Stonier³ for another bacteriocin. Heip *et al.*⁴ have outlined a partial purification procedure for agrocin 84 from solid media, but the degree of purification was not established and no structural studies were reported.

We have found that isolation is greatly facilitated by culturing a rough colony variant of strain 84 in a defined liquid medium. After a 10,000-fold purification of the agrocin 84 had been achieved by gradient elution techniques involving charcoal adsorption and anion exchange chromatography on DEAE Sephadex, a correlation between biological activity and the presence of a nucleotide with an absorption spectrum maximum at 264 nm was noted and found to hold for all the chromatographic and electrophoretic systems which have been investigated.

After further purification by electrophoresis on glass-fibre filter paper agrocin 84 has been characterised as an adenine nucleotide with the characteristic spectra shown in Fig. 1a. The purified agrocin contained adenine, phosphorus, 3'-deoxy-D-arabinose and D-glucose in the molar ratio of 1:2:1:1. It also seems to contain an as yet unidentified reducing sugar acid, and the presence of other constituents cannot be excluded at the present stage of the purification. An upper limit for the molecular weight of the triethylammonium salt is set at 1,100 ± 100 by an $A_{1\text{cm}}^{1\%} = 180$ and an ϵ value of 19,860 at 264 nm and neutral pH for the purest solid samples obtained to date.

By defining an arbitrary agrocin 84 unit as that amount of agrocin necessary to inhibit totally a zone of radius 1.0 cm within a lawn of pathogenic indicator strain 24, using the

Stonier³ bioassay, we have found at pH 7, that one 264 nm absorbance unit of the purified agrocin 84 contains $8 \pm 1 \times 10^3$ agrocin units. On a molar basis this corresponds to 1.6×10^{11} units mol^{-1} .

The infrared spectrum of agrocin 84 showed the presence of a P=O stretching frequency at $1,225 \text{ cm}^{-1}$ and the absence of simple carbonyl groups and the pyrophosphate grouping. The electrophoretic mobility/pH profile indicated the absence of any secondary dissociation corresponding to a phosphate monoester.

Glucose and 3'-deoxy-D-arabinose were identified as the main carbohydrate components of an acid hydrolysis of agrocin 84 by chromatographic and electrophoretic comparison with authentic samples; the pentose reference sample was obtained from a crystalline sample of methyl 2,5-di-O-acetyl-3-deoxy- β -D-threo-pentofuranoside by the procedure of Casini and Goodman⁸. Alkaline hydrolysis (1 M NH_4OH , 100°C 30 min) of the agrocin 84 produced a crystalline adenine nucleoside containing this unusual pentose, with the typical ultraviolet spectral behaviour ($\epsilon_{\text{max}}^{260.5} 14,300$, pH 12.55; $\epsilon_{\text{max}}^{260.0} 14,400$, pH 6.92; $\epsilon_{\text{max}}^{258.0} 14,200$, pH 1.06) for a 9-substituted adenine nucleoside.

It is now a decade since the corresponding 'fraudulent' nucleoside 9-(3'-deoxy- β -D-threo-pentofuranosyl) adenine (structure III, R = H) was first synthesised by Martinez, Lee and Goodman⁶ in the course of a cancer chemotherapy programme. Although the original sample is no longer available, the required nucleoside has been synthesised by desulphurising the penultimate intermediate 9-(3-S-ethyl-3-thio- β -D-arabinopentofuranosyl) adenine (structure III, R = $\text{CH}_3\text{CH}_2\text{S}$ -) with Raney Nickel.

The synthetic nucleoside (structure III, R = H) and the nucleoside from the alkaline degradation of agrocin 84 were found to be indistinguishable in all the properties examined, including melting point, mixed melting point, optical rotation, paper and thin-layer chromatographic systems, electrophoretic systems, gas chromatography of the trimethylsilyl derivatives, mass spectra of the nucleoside and its silyl derivative, ultraviolet spectral behaviour, and infrared spectra.

With the establishment of the structure of the nucleoside fragment of agrocin 84 as structure III (R = H), attention was next directed to the nature of the substitution of the nucleoside, which would result in the ultraviolet spectra of the intact agrocin as shown in Fig. 1a. Four properties of the agrocin which are particularly relevant, are first, the initial bathochromic shift in the absorption maximum from 264 nm to 270 nm on acidification (Fig. 1a); second the relatively rapid hydrolysis of the agrocin in 0.1 M HCl at room temperature to produce a nucleotide with an absorption maximum at 260 nm at neutral pH values and whose ultraviolet spectral shape and behaviour are similar to other 'normal' 9-substituted adenine nucleotides; third the extreme thermal lability (110°C , pH 7, 15 min) of agrocin 84 at neutral pH to produce a second nucleotide with an absorption maximum at 260 nm; and finally the room temperature hydrolysis of agrocin 84 in alkali (1 M NH_4OH , 25°C , 3 h) to produce a third nucleotide, which is thermolabile, contains D-glucose, has an absorption maximum at 264 nm, and is similar in ultraviolet spectral behaviour to the intact agrocin. These three nucleotides are readily resolved from agrocin 84 and one another, by electrophoresis in 0.05 M sodium tetraborate buffer at pH 9.4.

The reported^{7,8} bathochromic shifts for 6-N-acylated 9-substituted adenine nucleoside derivative and the known thermolability and acid lability of phosphoramidates led us to postulate the presence of a 6-N-phosphoramidate grouping. Model 6-N-phosphoramidates of adenosine were therefore synthesised from 2',3',5'-tri-O-acetyl adenosine⁹ and the appropriate phosphate, using dicyclohexylcarbodiimide in pyridine as the condensing agent, under conditions analogous to those described by Tener¹⁰ for the condensation of cyanoethyl phosphate with 2'-deoxyadenosine. Figure 1b shows the spectral characteristic of 6-N-(cyanoethylphosphoryl) adenosine (structure II, R = CNCH_2CH_2 -). At pH 5.0 the spectral

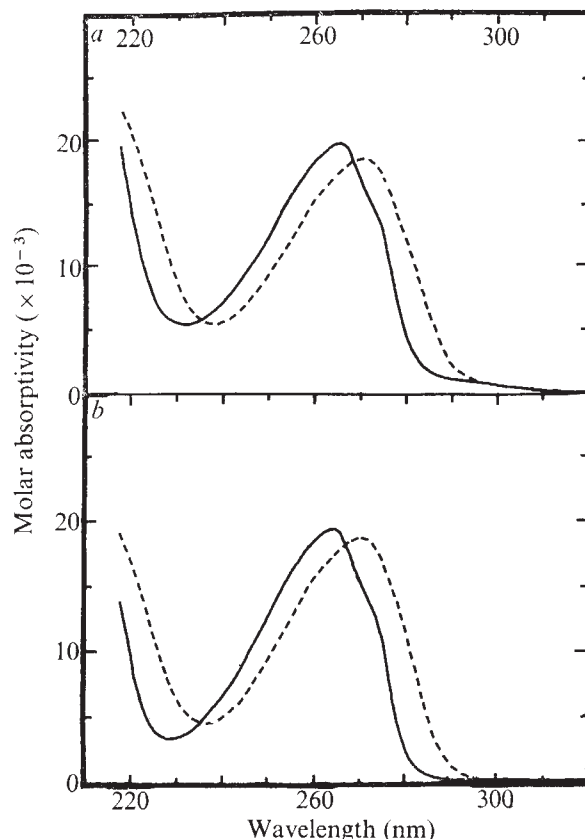


Fig. 1 a, Absorption spectrum for agrocin 84 at pH 1 (---) and 7 (—). b, Absorption spectrum for 6-N-(cyanoethylphosphoryl) adenosine (structure II, R = CNCH_2CH_2 -) at pH 1 (---) and 7 (—).

behaviour of the monoprotonated 6-N-(hydrogen phosphoryl) adenosine (6-N-AMP) (structure II, R = H) with an absorption maximum at 264 nm, ϵ , 19,500, resembles its cyanoethyl precursor shown in Fig. 1b. The non-protonated species of 6-N-AMP in 0.1 M sodium hydroxide, shows a shift to 267.5 nm, ϵ , 19,800.

The striking resemblance between the spectra of agrocin 84 in Fig. 1a and the 6-N-(cyanoethylphosphoryl) adenosine in Fig. 1b leaves little doubt that agrocin 84 contains the hitherto unrecognised 6-N-phosphoramidate linkage to adenine as shown in structure I, where R¹ is at present considered to contain glucose. R² and R³ represent hydrogen and a second phosphorylated carbohydrate moiety or vice versa.

The current investigation has firmly established the structure of the 'fraudulent' nucleoside fragment and the unique aspect of the phosphoramidate linkage is clearly indicated. If we accept the definition of a bacteriocin as simply 'an antibiotic produced by strains of bacteria and in general active on some other strains of the same species or related species'¹¹ and do not further restrict it to being 'colicin-like' as mentioned by Reeves¹¹, then this agrocin 84 should probably be classed as a new type of bacteriocin.

In conclusion, we point out that current attempts to explain all biochemical phenomena involving adenine nucleotides solely in terms of the 2',3', and 5'-phosphate esters may be less than adequate and a thorough search for other labile phosphoramidates such as the naturally occurring agrocin 84 and the synthetic 6-N-AMP reported here, with their predictably high phosphoryl transfer potentials, should be rewarding.

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Three-dimensional packing of collagen in bone

BONE collagen, which forms approximately 90% of the organic matrix of bone, differs from rat tail tendon and skin collagen in its solubility properties¹. Using low-angle X-ray meridional photographs and electron microscopy, however, I have found that the banding pattern of the collagen fibrils of bone is similar to that in other collagenous tissue, but that low-angle X-ray equatorial pattern could not be found.

Many explanations^{2,3} have been proposed for the banding pattern of collagen fibrils (Fig. 1) based on the low-angle meridional pattern of X-ray photographs and on electron micrographs. But to describe the packing of tropocollagen molecules within the collagen fibrils in bone, which can be deduced from low-angle equatorial pattern of X-ray photographs, it is necessary to take account of the presence of needle-like mineral particles aligned along the collagen

Fig. 1 Electron micrograph of section of demineralised foetal bone ($\times 11,000$).

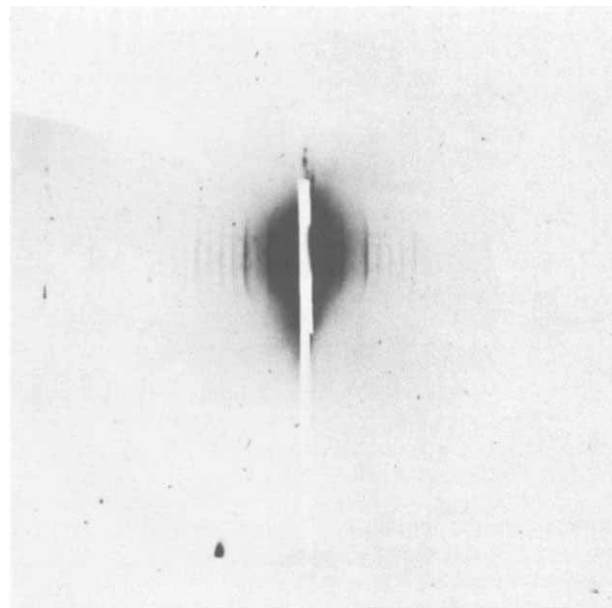


Fig. 2 Low-angle X-ray photograph of demineralised mature bone in water taken at Birkbeck (resolution shows only in one dimension).

fibrils and the lack of orientation of collagen fibrils with respect to one another.

Low angle meridional patterns of bone collagen in its mineralised and demineralised form were recorded by using a low-angle X-ray apparatus of the Holmes-Huxley type (mirror and crystal-monochromator)⁹ (Fig. 2), but no low-angle equatorial pattern could be found even after 3 d of continual exposure to X irradiation.

The experiment was repeated at DESY (Deutsches Elektron-Synchrotron) in Hamburg because synchrotron radiation is a more powerful source of X irradiation, and because in that situation⁸ meridional and equatorial patterns can be recorded simultaneously. Low-angle X-ray photographs of calcified turkey tendon (as a reference) and

Fig. 3 Low-angle X-ray photograph of turkey leg tendon (dry) taken at DESY.

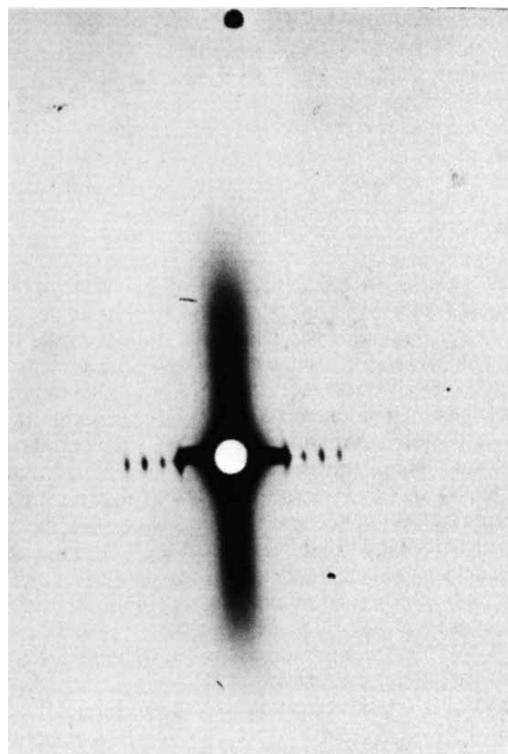


Table 1 Distances and intensities of meridional low-angle X-ray lines (average)

	No.	Bone (synchrotron) (Å)	I	Bone (Birkbeck) (Å)	I	Tendon (synchrotron) (Å)	I
—	1	—		645	S	621	VS
676	2	338	S	343	VS	365	M
678	3	226	VS	222	VS	232	VS
672	4	168	W	162	W	173	S
685	5	135	S	132	S	141	S
672	6	112	W	111	W	117	S
679	7	97	M	95	M	101	W
680	8	85	W	32	W	87	W
675	9	75	M	73	M	78	W
680	10	68	VW	66	VW	70	VW
682	11	62	VW	60	VW	64	VW
660	12	55	VW	54	VW	—	—

I, intensity; S, strong; VS, very strong; M, medium; W, weak; VW, very weak.

demineralised and mineralised human cortical femoral bone were used (Figs 3, 4 and 5).

The distances and intensities of the meridional low-angle X-ray lines (rings) shown in Table 1 indicate that there is little difference between the distances and intensities of the various orders of the low-angle meridional lines (rings) in tendon and bone. The main difference between the X-ray

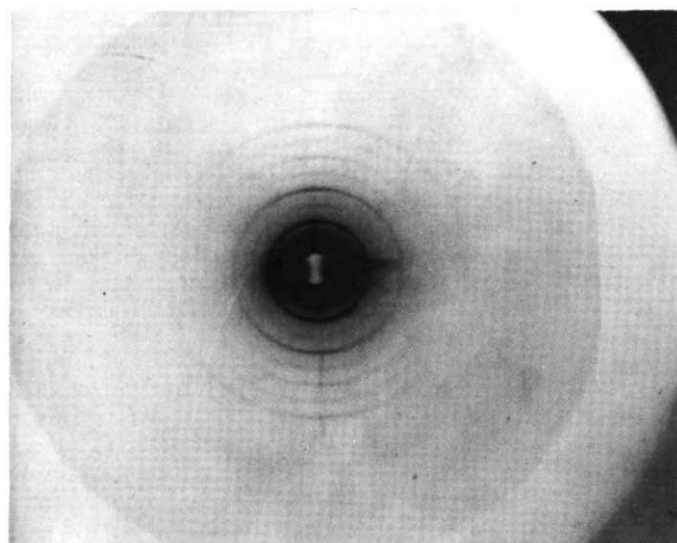


Fig. 4 Low-angle X-ray photograph of demineralised mature bone in water taken at DESY.



Fig. 5 Low-angle X-ray photograph of mineralised mature bone in water taken at DESY.

photographs is that in bone the meridional lines are extended to form full circles. Thus there is no low-angle X-ray equatorial pattern and it is not possible to deduce the three-dimensional packing of the collagen molecules within the fibrils or even to state that there is a three-dimensional order, as seen in tendon. The photographs also show that there is a preferred orientation of the collagen fibrils at about 22–23° from the c axis and that the continuous scatter due to the short axis of the mineral particles (Fig. 5) is perpendicular to the zone of preferred orientation of the collagen fibrils. This suggests that the long axis of the mineral particles lies along the long axis of the collagen.

These results show that the ultrastructure of the collagen fibrils of bones and other collagenous tissues is similar when examined by electron microscopy and low-angle X-ray diffraction (meridional pattern).

There are two possible reasons for the failure to observe a low-angle equatorial pattern: either the lack of orientation (full rings) conceals the equatorial pattern or there is

no equatorial pattern and hence no three-dimensional ordering within a collagen fibril in bone. In either case low-angle X-ray pictures alone cannot show whether three-dimensional packing exists within the collagen fibrils in bone, and the models for rat tail tendon, based on the same technique^{4–7} are not directly applicable to bone collagen.

This work was completed while I was at Birkbeck College, London. I thank members of the Department of Crystallography and also EMBO for a fellowship and the use of facilities at DESY.

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matters arising

Energy analysis of wave-power and wind-power systems

MUSGROVE¹ paints a depressing picture of the energy analysis of wave power but seems to have largely overlooked the literature on its progress^{2,3}. The model tested by Swift-Hook *et al.*⁴ was of amateur construction as I can personally vouch. Since then the generous support of the Department of Industry has allowed developments which point to an optimum duck diameter of about 15 m instead of the 50 m suggested by Denton *et al.*⁵. This reduces the displacement to 200 tonnes per metre instead of the 2,000 quoted by Musgrove.

There is a further respect in which Musgrove's analysis is incorrect. He assumes that we intend to use steel, but the high energy content of steel is a strong incentive to use as little of it as possible. Concrete requires only one seventh of the energy of steel⁶ and it is suitable for much of the structure.

I am keenly aware of the many difficulties facing wave power and so I am hesitant to accept his prediction that (with correct input data) the energy repayment period will be 16 years divided by 70 which is eighty or ninety days.

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MUSGROVE REPLIES—In my recent letter¹ I estimated that the energy recovery period of a rocking float wave power system would be about 16 years. Salter believes that this may be greatly reduced. In particular he suggests that reducing the diameter from about 50 m to about 15 m will reduce the energy recovery period by a factor of ten; the use of concrete instead of steel will then, he suggests, reduce the energy

recovery period by a further factor of 7.

My original letter did consider the effect of reducing the diameter of rocking float systems but, as I pointed out, reduced diameter gives reduced efficiency and an increased ratio of structure weight to displacement; the gain is therefore appreciably less than Salter suggests. Moreover, the simple calculation, which in my letter gave an energy recovery period of 16 years, was based on a wave power system sited at Station India, 700 km out in the

that of steel, but no one has yet built a concrete supertanker. I sincerely hope that the use of concrete will allow a seven-fold reduction in the energy recovery period of rocking float wave power systems. However, even if this can be achieved, it should be recognised that wind power systems have the potential to provide as much energy as wave power systems, and present much less formidable development problems.

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¹ Musgrove, P. J., *Nature*, **262**, 206–207 (1976).

² Denton, J. D., *et al.*, *CEGB Res.*, **2**, 28–40 (1975).

[Power outputs for different sizes of installation have been given by Molli-son *et al.* in *Nature*, Vol. 263, pp. 223–236, September 16, 1976. Ed.]

Use of mechanical barriers instead of guard rows in field experiments

A TECHNIQUE in which vertical strips of Vinyl copolymer foil were used to separate experimental plots in a large field experiment on fruit trees was reported in *Nature* in 1962¹. The experiment, involving different water and nitrogen regimes, reached a successful conclusion in 1975, and during 1976 an examination was made of the foil and of the behaviour of the roots when they encountered it. The foil was intended to serve as a barrier to root extension across plot boundaries in place of guard rows of trees. This was achieved surprisingly well; roots were contained without any penetration of the foil and remained at the depth at which they had met the barrier and turned.

Since the barriers were laid to a depth of 0.86 m, root exploration downwards might have led to serious poaching from adjacent plots. However, this does not appear to have occurred even after 15 years.

In this experiment the need for 4.4 ha of land with guard trees was reduced to 1.9 ha; this saving of experimental land together with greatly reduced risks of inter-plot variability and the satisfactory containment of treat-

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Atlantic, where the annual average power flux is 77 kW m⁻¹. A more typical location is Seven Stones, off Land's End, where the annual average power flux is 26 kW m⁻¹. At this location a 50 m diameter rocking float system would have an energy recovery period of approximately 47 years, and lack of alignment between the wave power system and the incident waves will tend to increase this figure. Reduced diameter does however give some reduction in the energy recovery period and hence I concluded that overall one could expect to achieve an energy recovery period of about 16 years.

This estimate does assume steel construction, consistent with the assumption, made by Denton *et al.*², that the cost per unit displacement of rocking float wave power systems will be comparable with that of existing supertankers. The specific energy content of concrete is, I agree, much lower than

ments and roots at a cost considerably below that of concrete barriers¹ probably afford the technique a wide application.

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¹ Goode, J. E., and Marchant, W. T. B., *Nature*, **195**, 410 (1962).

Plutonium and americium in fish

PENTREATH and Lovett¹ have presented data on $^{239+240}\text{Pu}/^{238}\text{Pu}$ ratios in plaice which cause us some concern in the light of what we have recently reported on the probability of biological discrimination between different plutonium isotopes². Although not discussed in their report the ratios in different fish tissues from any one composite sample seem to show approximately two- to threefold differences in the uptake behaviour of ^{238}Pu and $^{239+240}\text{Pu}$, depending on the time of year. As no consistent trends among the ratios in the various tissues seem evident, we question the significance of these differences. Counting errors associated with the $^{239+240}\text{Pu}$ measurements run typically about 20% (range 4–47%). The errors for the ^{238}Pu measurements are not reported but undoubtedly are higher due to the lower levels of ^{238}Pu in the samples. Furthermore, where counting error for $^{239+240}\text{Pu}$ is the least (for example, gut contents), the ratios are more consistent and tend to approach a mean value of 4.4, which is in good agreement with the ratio (4.3) measured in surface sediment² collected from the same general area in July 1975. In fact, if the same relative counting errors reported for the $^{239+240}\text{Pu}$ measurements are assigned to the corresponding computed ^{238}Pu values, comparison of the mean values of the ratios for all tissues from each collection do not indicate differential behaviour between the isotopes. We propose that if the counting errors associated with both the ^{238}Pu and $^{239+240}\text{Pu}$ measurements were propagated, the resultant errors about the ratios would indicate that the apparent differences in plutonium isotope behaviour in certain tissues suggested by their data are not, in fact, significant.

Furthermore, we question the advisability of computing concentration factors for plutonium and americium in plaice in which only filtered seawater concentrations of the actinides are used. In this case the impression given is that ^{241}Am accumulates in plaice muscle fivefold over that of $^{239+240}\text{Pu}$. In another study³, concentration factors in worms based on total

plutonium and ^{241}Am in Windscale sediments indicate that the accumulation of plutonium is favoured over that of ^{241}Am by a factor of two. The discrepancy could be due to a 'species effect'; however, it also may be simply an artefact of the different way in which the concentration factors were computed.

Summation of the monthly discharge data reported by Pentreath and Lovett gives an estimated integrated annual discharge ^{241}Am /plutonium ratio of about 1.4, which is not too unlike either that measured in the sediments³ (1.7) sampled in July 1975 or those that can be computed from their data for the various fish tissues (especially November 1975) and gut contents. These similarities suggest to us the possibility of a real ^{241}Am /plutonium ratio in water somewhat higher than 0.2 reported for ^{241}Am and $^{239+240}\text{Pu}$ in their filtered sample. One recent report⁵ states that 30% of the $^{239+240}\text{Pu}$ in their filtered samples. One recent report⁴ states that 30% of the $^{239+240}\text{Pu}$ in Windscale waters is associated with filterable particulates ($>0.22\text{ }\mu\text{m}$). While apparently no comparable published data for ^{241}Am exist, there are indications^{5,6} that ^{241}Am may be strongly particulate in seawater. We recognise the limitations involved in determining concentration factors; however, until more information is at hand concerning the bioavailability of both soluble and particulate forms of these elements, we believe that concentration factors based only on one fraction of the total element in water may be artificially high.

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¹ Pentreath, R. J., and Lovett, M. B., *Nature*, **262**, 814–816 (1976).

² Beasley, T. M., and Fowler, S. W., *Nature*, **262**, 813–814 (1976).

³ Beasley, T. M., and Fowler, S. W., *Mar. Biol.*, **38**, 95–100 (1976).

⁴ Hetherington, J. A., et al., in *Impacts of Nuclear Releases into the Aquatic Environment: IAEA-SM 198*, 193–212 (International Atomic Energy Agency, Vienna, 1975).

⁵ Volchok, H. L., et al., in *Assessing Potential Ocean Pollutants*, 27–63 (National Academy of Sciences, Washington, DC, 1975).

⁶ Murray, C. N., and Fukai, R., in *Impacts of Nuclear Releases into the Aquatic Environment: IAEA-SM 198*, 179–191 (International Atomic Energy Agency, Vienna, 1975).

PENTREATH AND LOVETT REPLY The errors associated with the ratios of $^{239+240}\text{Pu}/^{238}\text{Pu}$ were omitted in order to avoid a discussion on them. Fowler and Beasley are correct in assuming that most of them are not significantly different, as deduced from errors associated with counting. But there were one or two samples which did seem to be different and these are being investigated further. By and large, however, we are generally impressed as to their lack of variance in view of the widely

differing ratios which obtain in the Windscale area. Fowler and Beasley argue from the axiom of a constant ratio, assumed to be $\approx 4.3:1$. In fact the ratio in the Windscale discharge is not accurately known but seems to have declined from $\approx 20:1$ to $\approx 4:1$ in the last 10 years as deduced from measurements made on surface sediments¹. The ratio thus also varies with depth in the sediment, and this variance with depth differs from one area to another. The fish may therefore have been exposed to slightly different seawater ratios and to largely different ratios in ingested material. Because each organ may have a different biological half-time for plutonium, different ratios could be expected in the organs of some fish. This is rather speculative, however, and it would be very difficult to demonstrate in individual fish and could only explain large ratios.

With regard to the comments concerning the calculation of concentration factors, these calculations were made using the only data available at the time—filtrate sea water samples taken from the same area and at the same time as the August fish samples. These data were kindly proffered by a colleague and we thought the calculations made with them would be of interest; we still do. In doing so, however, we wished neither to commend nor condemn the use of filtrate seawater values for the general calculation of concentration factors. There are as many arguments against using total seawater values—such as the variability in particulate matter in shoreline areas—as there are for their use. The views on this subject of one of us (R.J.P.) are discussed at length elsewhere². For the Windscale area the greatest sources of error for plaice are likely to be, as stated in our paper, the variable discharge rate and the movements of the fish into and out of the area close to the discharge pipe.

Finally, with regard to the amounts of ^{241}Am relative to those of plutonium, we remarked that the concentrations of the former may well have been influenced by a large release at the end of 1974. It is of interest that in samples taken in February 1976 the ratio was $>2:1$ in the majority of organs whereas that of the discharge during the previous six months has been $<0.5:1$.

In short, therefore, the principal objective of the paper was to give some indication of the concentrations of plutonium and americium in plaice in comparison with the amounts of these radionuclides discharged. The points raised by Fowler and Beasley were not discussed as we considered it more advisable to obtain further data rather than attempt to over-interpret those few data which we had already obtained.

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¹ Hetherington, J. A., in *Environmental Toxicity of Aquatic Radionuclides: Models and Mechanisms*, 81–106 (Ann Arbor Science, Michigan, 1976).

² Pentreath, R. J., in *Oceanogr. mar. Biol. A. Rev.*, **15** (in the press).

reviews

Ethology of ethology

John R. Krebs

Growing Points in Ethology. By P. P. G. Bateson and R. A. Hinde. Pp. viii+548. (Cambridge University: Cambridge, 1976.) £16.

My reaction on perusing this volume was one of optimism for the future of ethology. An appreciable proportion of the eighteen contributions to this symposium, held in June 1975 to celebrate the 25th anniversary of the Madingley Field Station (Cambridge, UK), discuss interesting new ideas and approaches or bring new evidence to bear on areas of current excitement. In comparison with a parallel volume produced by Madingley ethologists and their associates 15 years ago the theoretical arguments are less concerned with semantics and classification, and more with real attempts to seek underlying generalisations. It is also comforting to note that most of the major areas of interest have changed considerably in 15 years. The editors, who have done an excellent job in writing brief discussions to summarise, coordinate, and occasionally criticise the individual essays, have arranged the volume along the lines of Tinbergen's classical 'four questions'—causation, function, evolution and development. There is also a considerable section on 'human ethology'.

In spite of the risk of being accused of chauvinism, I must state that I found two of the most original articles to be those by Dawkins and McFarland, both of whom have tried to develop new approaches to constructing general theories of behaviour. Dawkins argues that explanatory principles of the mechanisms of complex behaviour should come at the purely behavioural, rather than physiological level. After the burial of some early ethological models of motivation this sort of approach has not been much developed. But since the complete physiological description of complex behaviour is a distant and perhaps not even fruitful goal, "software" explanations, as Dawkins calls them, are obviously of crucial importance. Dawkins goes on to reason on functional, "neuroeconomic" grounds that evolution will tend to develop hier-

archical control mechanisms of behaviour, and that this should be a general principle of behavioural organisation. He also describes various analytical tools for detecting hierarchical properties of overt behaviour.

McFarland also argues for software explanations of behaviour, but from a different standpoint. He suggests that even if one knew all the physiological mechanisms underlying, say, feeding and sexual behaviour in the dog, one would still not be able to look at a dog and predict whether it will eat or copulate next, because this will depend on how the dog balances the two tendencies against one another in making a decision. The decision rules can only be analysed at the behavioural level, and the problem is essentially an ecological one, because the animal will have been designed by evolution to make ecologically sensible (adaptive) decisions. McFarland then outlines a theoretical method for tackling the difficult problem of how a cost-benefit analysis of decision making can be achieved. The aim of the theory is to predict the optimal decision rules, which can then be tested against the actual data. Although this type of approach has been used in the ecological literature on foraging behaviour, this is the first attempt to apply the rationale to many different behaviour patterns at the same time.

The recent surge of interest in kin selection theory, and especially in its role in the evolution of altruistic behaviour, is reflected in the articles by Clutton-Brock and Harvey on primate social groups, and by Bertram on lion prides. The former paper is a useful discussion of aspects of primate social organisation, such as pair bonding, altruism, aggression and sexual dimorphism, in the light of current evolutionary thinking. Bertram takes the analysis of kin selection further (to my knowledge further than any other vertebrate study, with the possible exception of the work on group territorial birds) by calculating the degree of relatedness (r_0) between the various members of a lion pride. Bertram then discusses whether or not kin selection can account for various altruistic acts in the pride. For example, mothers

suckle one another's cubs, even though the degree of relatedness between a mother and these cubs is very low, about 0.075. So kin selection is not likely to have played a major role in the evolution of this trait. On the other hand, the observation that males more than females are tolerant of cubs feeding at kills, fits in well with the calculated differences in r_0 : 0.31 for males and cubs, and 0.18 between females and an average cub. Clutton-Brock and Harvey discuss an analogous example of dominant Japanese macaques tolerating subordinates according to how closely related they are.

The section on human ethology contains two particularly useful articles (by Hinde and Hinde, and Blurton Jones) in which the interface between ethology and social psychology (in the former) and anthropology (in the latter) are explored. Hinde and Hinde make a convincing case that it is now possible to study on a truly scientific basis the qualitative nature of inter-individual relationships, a problem from which most ethologists would have recoiled in horror ten years ago. Blurton Jones urges that the deductive approach, based on natural selection theory, used for explaining and predicting social organisation in animals, should also be applied to human cultures. Peculiar cultural customs should not be regarded as quirks of chance drift, fascinating and inexplicable, but viewed as ecological adaptations. In this way, he argues, it should be possible to develop a coherent theory of cultural differences.

Returning to my opening comment, this volume shows convincingly that ethology is not, as E. O. Wilson has prophesied, about to meet its death at the hands of neurophysiology and sociobiology. There is still a remarkably exciting atmosphere resulting from the blending together of causal, developmental and functional questions about behaviour, that still characterises ethology. □

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Hydrodynamics and water waves

An Introduction to Hydrodynamics and Water Waves. By Bernard Le Méhauté. Pp. viii+323. (Springer: Berlin and New York, 1976.) DM 60.60; \$24.80.

AN obvious question to ask about this book is: what are the special contributions which it offers to an already well endowed literature on hydrodynamics and water waves? The declared aim of the book is to provide a text based on a set of lecture notes for engineering students, that introduces the mathematical aspects of fluid mechanics with explanations of physical meaning to help practising engineers to read mathematical texts and keep pace with new developments reported in scientific journals. It is intended, moreover, to have a wide range of application and in particular to appeal to "students of coastal, hydraulic and civil engineering, naval architecture, physical oceanography, marine geology and sedimentology".

Although it inevitably covers much well trodden ground, it fulfils its aims and has much to offer in clarity of explanation and in its orderly presentation of a broad selection of theoretical material. It may be disappointing, however, to those seeking a close contact with current engineering developments.

The text is divided into three main parts concerned, respectively, with the derivation of the basic equations of fluid motion, mathematical treatment of the equations, and water wave theories. There are two appendices, one dealing with wave spectra and associated statistical properties, and the other with problems of scale modelling.

Examples of clarity include well illustrated accounts of the distinctions between streamlines, stream tubes, streak lines and particle paths and of the significance of dilatation, shear and rotation in interpreting the kinematic equations. I specially commend the many well organised tabular summaries of key details and formulae which students will surely find very helpful and which offer convenient working references for practising engineers. Students should also appreciate the selection of illustrative problems at the end of each chapter for which answers are given in the back of the book. Although prominence is given to development of basic theory relevant to a broad treatment of water waves, material is included on flow in porous media and pipes, and on open channel hydraulics. Its claim to appeal to students in a wide range of professional fields therefore seems justified.

Although the book offers a clear and broadly based conceptual framework for the study of theoretical fluid dynamics the degree of contact with current engineering technology may be disappointing to those with a more practical turn of mind. A particular feature, for example, that I find rather frustrating is the almost total absence of cited references. There are lists called "References" at the end of each part but these might more appropriately be headed "Recommended background reading", since there are hardly any explicit references to these works in the text. The reader is therefore repeatedly tantalised by the introduction of complex ideas such as tensors, conformal mapping, ship waves and diffraction theory which are only very briefly discussed without mention of any references for further study.

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Acetylcholine, the model transmitter

Biology of Cholinergic Function. Edited by A. M. Goldberg and I. Hanin. Pp. xiv+716. (Raven: New York, September 1976.) \$42.

BOOKS for biomedical scientists often promise more than they actually provide because the titles of convenience they bear have been conjured up by ingenious editors who wish to confer (on a disparate collection of symposium papers, for instance) a suggestion of cohesiveness and completeness where none exists. It is a pleasure to be able to declare at the very outset that *Biology of Cholinergic Function* escapes this criticism: it really does provide everything—perhaps more than—its title offers. I find it difficult to believe that anyone who buys the book will be disappointed with his purchase, even though his intellectual enrichment will be bought at the cost of economic impoverishment to the tune of £26 at this morning's rate of dollar exchange.

Neurotransmission processes attract a good deal of attention nowadays, and deservedly so, but in the midst of our animated discussions concerning such lively topics as monoamines and mental illness, the reawakening of interest in the possible transmitter roles of ATP, substance P and histamine and the enthusiasm in many quarters in an increasing range of transmitter amino acids, it is easy to ignore acetylcholine, the earliest, the best authenticated and the most pervasive mediator of them all. Cerebral control systems rarely

lack cholinergic synapses even though recent attention may seem to have been largely focused on their more immediately glamorous non-cholinergic components. Work on acetylcholine has, however, continued apace during the years when other substances have occupied the centre of the stage; and no worker in, or student of, the field of chemical transmission can afford to ignore it. It is conveniently assembled for him within the substantial covers of this book.

This massive compilation (more than 700 pages) is encyclopaedic in scope with exhaustive and up-to-date surveys of the literature (papers published as recently as 1975 are referenced) and of recent experimental findings. The main body of the text—18 chapters in all—discusses acetylcholine metabolism and actions in vertebrates and invertebrates, at subcellular and at whole organ levels, at peripheral and central sites, in muscle and in nerve. Those who champion the claims of non-cholinergic transmitters would do well to pay particular attention to the chapter that discusses the interaction of cholinergic and non-cholinergic transmitter processes and to the group of four chapters that together form a section on cholinergically linked diseases. The book is enriched for the laboratory worker by the inclusion of two appendicular tables. One of them, nineteen pages long, provides information concerning the acetylcholine content and cholinesterase activity of the tissues. It lists the results of all the studies that have worked their way through into the literature. The other table, only two pages shorter, does the same for the actions of drugs on the acetylcholine system. These tables are described in the preface as compendia of 'cholinergic parameters' but, this semantic monstrosity apart, the literary style of the book as a whole is tolerable if not exactly memorable.

Multiauthor texts are prone to exhibit irregularities in style and in depth of treatment and to suffer from omissions and repetitions that irritate the busy reader. This volume cannot be faulted on these grounds because the twenty-nine contributors worked together as a team under the guidance of editors who clearly took their duties seriously. All twenty-nine authors live in the United States but this does not unfairly bias the book towards American workers and attitudes. I hope that this judgment is not unduly influenced by the fact that my own modest contributions to the subject of the book are not ignored.

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Great men and the mainstream

History of Mankind: Cultural and Scientific Development. Volume 5. Parts I-IV: The Nineteenth Century. Edited by C. Moraze. Pp. 1,394. (George Allen and Unwin: London, 1976.) Parts I-II: £15; III: £15; IV: £15.

THIS curious 'volume' appears in three substantial tomes. The various chapters in it are written by various authors, or teams of authors; many of these are based in French institutions (other European countries being little represented) while others are from the USA, the USSR, and other nations. Sometimes the author is acknowledged at the top of his contribution, whereas for others one must search among the small type at the beginning of the first tome; no credit seems to be given to the translators, who have produced smooth versions of what were presumably papers for the most part not written in English.

The work is curious for various reasons; first because it was clearly written many years ago, as indeed is admitted in the preface where a date of "before 1960" is given. We are therefore getting a warmed-up hash, many parts of which have become obsolete long since; and it is even a half-baked hash, since the bibliographies have been omitted. By this tardy and incomplete publication, justice is hardly done to the various contributors, whose reputations will not be advanced by association with this project.

Even odder is the fact that the chapters were evidently sent to referees in various countries for comments; but were then published in their original form, with the referees' comments appended. Occasionally the author has commented on these comments, and his remarks also appear. The chapters are therefore accompanied by hostile annotations, often all too justified: but because of the omission of the bibliographies, we find dogmatic statements by the author and commentator; and cannot choose between them since we do not know on what evidence they are based. To publish an historical work without a proper account of the sources used is like publishing a scientific paper without any experimental details. Even for the illustrations, we are not always told where and when they were first published, although we are told which institutions made them available.

History was the province of a muse, and one might hope that a work of history would be a work of art; but if so, one would be disappointed with this volume, in which few of the chapters are written with any verve. Some con-

tributors have an axe to grind—this can be a great help in an historical essay—but this usually leads only to the dullness of Marxist terminology; whereas others are preoccupied with the hopeless attempt to leave nothing out.

It is the first tome, dealing with the history of science and technology, to which readers of *Nature* are probably most likely to turn; and we can therefore examine it in more detail. In the introduction by the editor, abstractions chase one another in what is a completely schematic and impressionistic essay, being neither narrative nor detailed case study. When we come to mathematics and astronomy, we find the account is completely 'internal' and is moreover full of sentences of the kind: "Tom, Dick, and Harry also made important contributions to this field". The Russian referee made the point that any social dimension is completely lacking; and the same can be said of the other writings on the physical sciences, those on medicine and the life sciences being better though clearly out of date.

The narratives concentrate on great men and on the mainstream, and tend to lapse into the passive voice; there is little feeling that science is carried on by people called scientists, and that an important part of its history is the study of their collective behaviour. It was in the nineteenth century that science became a profession; that science became specialised, with the general journals of the academies being supplemented by those of societies catering for one science, or part of one; that formal courses in the universities leading to first and higher degree began; and that governments and industries began to employ scientists in large numbers as new legislation and new processes were introduced. This is not apparent from these internal accounts; which is not surprising, because the emphasis in the history of science has shifted in the past twenty years or so, away from accounts of how we got to where we are now, often written by superannuated scientists, towards attempts to recover how past science actually worked and what people were trying to explain or control. These essays are monuments, of varying quality, to an elderly and amateurish tradition; the most interesting is the critical note on the history of technology by Melvin Kranzberg.

In general, therefore, it is hard to see who would ever use this monument to international cooperation, and harder to see who one would recommend to use it.

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Light-emitting diodes

Light-Emitting Diodes. (Monographs in Electrical and Electronic Engineering.) By A. A. Bergh and P. J. Dean. Pp. viii+591. (Clarendon: Oxford; Oxford University: London, 1976.) £22.

THE general public first became aware of light-emitting diodes (LEDs) when pocket calculators made their dramatic entry on to the market. The miniature calculator was in fact made possible by LEDs, although the more recent development of reliable passive displays, such as liquid crystal devices, would eventually have filled the gap. For many years preceding this sudden appearance, LEDs were the subject of intense research in solid-state laboratories in many parts of the world.

The history of this period is an exciting one, with its full share of disappointments and surprises, and the authors of this book have played an important part in promoting the theory and the technology of the subject. It is disappointing that, contrary to early expectations, conversion efficiencies from electrical energy to visible light remain low in spite of the massive effort put into improving them, so the rapid technical advance of the 1960s and early 70s has now slowed down. The fact that LEDs have now found wide commercial applications, makes this a period of stability and therefore a most appropriate time for the appearance of this comprehensive work on the subject.

Nearly half of the book is devoted to a deep and detailed account of the theoretical aspects of carrier injection and the factors affecting the efficiency of light generation, most particularly in the III-V compounds. There follows a good account of the interesting field of up-converters (infrared to visible)—phosphors which seem to defy the basic rules of phosphor physics. Even though this technique has not found commercial application, it may prove of great importance if solid-state lasers are developed with output wavelengths more closely matched to the excitation band of the few available phosphors.

The remainder of the book is devoted to the technology, design and application of LEDs and associated devices, and here again the treatment is detailed and comprehensive. A minor disappointment (perhaps inevitable if the book is to be constrained to a reasonable length) is the treatment of photometry. Although a whole chapter is devoted to this subject, and many aspects are enlarged on in the chapter on LED design, the results are put in the form of generalised equations. Photometry is such a jungle of defini-

tions, concepts and units that it is a permanent headache to the engineers trying to compare the merits of different light-emitters. An appendix containing some worked examples would have been very helpful.

The work is naturally devoted to the most theoretically tractable light-emitting devices, especially the III-V semiconductors, although there are brief discussions on SiC and ZnS. It is impressive in its deep treatment of the subject, and the enormous range of study is indicated by the collection of more than a thousand references. This makes it a valuable source-book and a necessary addition to the library of any physicist or engineer working in this field.

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Reptilian physiology

Biology of the Reptilia. Vol. 5: Physiology. Edited by C. C. Gans and T. S. Dawson. Pp. xv+556. (Academic: London and New York, October 1976.) £16.80.

THE preceding volumes of this encyclopaedic survey, which appeared between 1969 and 1973, were devoted to morphology; and herpetologists interested in physiology have had to await patiently the turn of their own speciality. The first of the physiological series, however, should not disappoint them. The literature on the subject is remarkably scattered because reptiles have been studied, not only in their own right but also as subjects for physiological and pharmacological assay. In the book under review, an attempt has been made to provide an overall summary orientated towards reptiles as a taxon rather than towards single topics such as metabolism, thermoregulation, or water balance. In this endeavour, the editors have enlisted authors who, in each case, have contributed signally towards elucidating the particular topics they summarise. The cast is all-American, but at least the book was printed and bound in Great Britain.

In their opening chapter, entitled 'Reptilian physiology: an overview', the editors emphasise the ecological aspects of environmental physiology. This is followed by a detailed discussion of 'Methods for the physiological study of reptiles' by Harry S. McDonald. Subsequent chapters are as follows: 'Metabolism' by Albert F. Bennett and William R. Dawson; 'Respiration: mechanics, control and gas exchange' by Stephen C. Wood and Claude J. M.

Lenfant; 'Circulation' by Fred N. White; 'Regulation of acid-base balance in reptiles' by Barbara J. Howell and Hermann Rahn; 'Osmo-regulation' by P. J. Bentley; 'Salt glands in reptiles' by William A. Dunson; and 'Renal function (with special emphasis on nitrogen excretion)' by William H. Dantzler. The volume concludes with a comprehensive index, but this does not seem to have been checked with quite the thoroughness usually characteristic of Academic Press.

Throughout, the reader will be struck by the heterogeneity of the Reptilia, which is reflected in their physiology. For instance, in many reptiles the salt gland is the major route for excretion of electrolytes; yet these glands have evolved independently, over and over again, in the process of adjustment to dry or saline environments. Great variation exists in the regulation of nitrogenous excretion, and this too may be related to habitat as well as to the end products of metabolism, the ability of the cloaca or bladder to regulate the volume and composition of the urine, and the ability to excrete ions by extra-renal routes. Similarly, metabolic rates are extremely variable, but little attention has yet been directed towards their functional correlates. The vast diversity of respiratory mechanics, lung morphology and gas transport mechanisms, is matched by comparable diversity in habitats, habits and levels of activity. The heterogeneity referred to above is thus clearly related not only to phylogenetic divergence but also to adaptive radiation within the various orders and suborders.

The search among living reptiles for intermediate evolutionary linkages with mammals and birds has diverted attention from the adaptive features and control mechanisms of the reptilian cardiovascular system. Yet, such features and mechanisms form an important part of the integrative physiology of the class. Reptiles have, potentially, a large contribution to make both to cellular biology and to ecology, and the future of reptilian comparative physiology should be linked closely with these areas.

Relatively few of the physiological processes discussed in this important volume have yet been investigated intensively, and any such studies comprise only a few species. Much work remains to be carried out before reptilian physiology can be understood really adequately. This book plays an important rôle in emphasising both what is, and what is not, now known.

J. L. Cloudsley-Thompson

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Molluscan delights

Pseudothecosemata, Gymnosomata and Heteropoda (Gastropoda). By S. Van Der Spoel. Pp. 484. (Bohn, Scheltema and Holkema: Utrecht, 1976.)

As once aptly observed by Dr Joel Hedgpeth, taxonomy is man's oldest profession, his "first task in the Garden was not to work for his living—that came after the Fall—but to name every beast of the field and every fowl of the air". Too soon interrupted in this, it has been left to succeeding generations to complete a task which increased in size with every new exploration, including the waters of the sea, and with every development in the capacity for magnification.

The significance of taxonomy was enhanced with the demonstration of the fact of evolution. What had been accepted as the works of a highly ingenious, if in certain aspects a by no means beneficent, Creator now appeared as the contemporary endpoints of innumerable, often interlocking, lines of evolutionary change. Taxonomy records the outcome of the most significant sequences of events that have occurred during the history of this planet.

To a marine malacologist it is difficult to imagine two more fascinating groups than prosobranch Heteropoda and opisthobranch Pteropoda (Euthecosomata, Pseudothecosomata and Gymnosomata) evolving independently as holoplanktonic gastropods. It is a delight to see with what elegance they are treated in this volume by Dr S. Van Der Spoel of the Institute of Taxonomic Zoology, Amsterdam. It succeeds an earlier one, in 1967, dealing solely with the Euthecosomata and is to be followed by a third concerned with the biology of all planktonic molluscs.

This surely is an ideal example of the manner in which the products of evolution should be treated with adequate description and synonymy of every species, accompanied by 166 beautifully drawn line figures—and how fully these exquisite animals merit such attention—followed by 81 maps showing world distribution. Appendices contain diagrams revealing the diversity of taxa in the different faunal centres with others assisting in the use of keys for identifying species difficult to separate, notably in the heteropod Atlantidae. Recent information about the Euthecosomata brings the earlier volume up-to-date, and a reference list of over 80 pages covers the entire literature.

Differing widely in origin, the

heteropods and pteropods differ also in distribution, a possible consequence of their different periods of origin. There are more heteropods in the Indo-Pacific but pteropods are commoner in the Atlantic. From this it is postulated that the former evolved in the greater oceanic area. But, being more thermophilous, they found difficulty in passing through colder southern waters into the Atlantic. They are not, however, absent in that ocean and nowhere do more spectacular species of both groups live together than in the Mediterranean, where this reviewer has found much pleasure in studying living representatives.

Dr Van Der Spoel's book is a work of combined scholarship and research and forms a permanent addition of the highest value to zoological knowledge. It should be contained in every museum, every marine laboratory and every University library. It is an indispensable work of reference to marine biologists, both applied and academic. It will be a delight to every malacologist.

C. M. Yonge

Sir Maurice Yonge, formerly Professor of Zoology at Bristol, then at Glasgow, is Honorary Fellow in the University of Edinburgh, UK.

Biology of Kinetoplastid flagellates

Biology of the Kinetoplastida. Vol. 1. Edited by W. H. R. Lumsden and D. A. Evans. Pp. xxvi+563. (Academic: London, New York and San Francisco, 1976.) £18.00.

THE parasitic members of the flagellate order Kinetoplastida of the phylum Protozoa have always attracted more attention than the free-living forms, and the species of two genera, *Trypanosoma* and *Leishmania*, which infect man, have attracted the most. It is easy to forget that these genera are only two out of sixteen or more, that many trypanosomes cause no harm to their hosts and that it might be possible to learn as much about trypanosomiasis and leishmaniasis from comparative studies on related forms as from the trypanosomes and leishmaniasis of man.

To many protozoologists, the literature is top-heavy with information on the Kinetoplastid flagellates of medical and veterinary importance and it is the intention of this multi-author volume (and its companion when it is published) to redress this imbalance and to present the Kinetoplastida as a whole. Difficult as this is to achieve, it

looks as if the two volumes between them will do just that.

This volume, for a number of reasons explained by the editors, is mainly concerned with trypanosomes, apart from the first two chapters (by K. Vickerman, and K. Vickerman and T. M. Preston) which deal with the diversity and cell biology of the kinetoplastid flagellates as a whole. Both these chapters contain a considerable amount of original thought and research material. There are also four comprehensive reviews of trypanosomes, of birds (J. R. Baker), of bats (C. J. Marinkelle), of primates other than man (C. J. Marinkelle) and of the subgenus *Herpetosoma* (D. H. Molyneux). These, with their well presented tables and check lists, will be invaluable sources of information for years to come.

Two of the remaining chapters cover the rapidly advancing fields of the biochemical aspects of taxonomy (B. A. Newton) and oxidative metabolism (I. B. R. Bowman and I. W. Flynn). Informative and useful as these are at present they will be even more useful when read in conjunction with the chapters on the taxonomy of the leishmaniasis and biochemical aspects of *Trypanosoma cruzi* which are promised in volume 2.

Two further chapters deal with the subgenus *Megatrypanum* (E. A. Wells) and *Trypanosoma rangeli* (A. D'Alessandro). Although their inclusion can be justified on the grounds that the information contained in them is not adequately summarised elsewhere, they are really too specialised to justify such extensive coverage in a book of this kind. The remaining two chapters on innate resistance to trypanosome infection (R. J. Terry) and antigenic variation (A. R. Gray and A. G. Luckins) are again rather specialised, and would have been better associated with other papers on immunity also due to be published in volume 2. In any case, antigenic variation could do with a rest from reviews for a year or so.

This is a most valuable work of reference but could have been so much better if the reproduction of the plates and electron micrographs had been of better quality, and if all the chapters had been updated from their original 1973 form as some of them have been. The publication of volume 2 is eagerly awaited. Perhaps it would not be too much to hope that each chapter in volume 1 will be briefly updated and that better quality paper will be used for the plates.

F. E. G. Cox

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Protein crystallography

Protein Crystallography. By T. L. Blundell and L. N. Johnson. Pp. xiv+565. (Academic: London, New York and San Francisco, 1976.) £17.00.

FOURTEEN years after the award of Nobel prizes to Crick, Kendrew and Perutz, this is the first textbook to be published on protein crystallography by an established practitioner in the field. Perhaps this indicates the compulsive preoccupation of the protein crystallographer with the mechanics of his work, but in any case the event is to be welcomed.

One of the attractions of protein crystallography is that narrow specialisations are not allowed. Without sound grounding in mathematics, physics, crystallography, inorganic chemistry, organic chemistry, biochemistry and biology, one will constantly run into problems one cannot evaluate. The experimenter needs in addition a smattering of knowledge of instrument design, high vacuum technology, electronics, chemical engineering and computer science. Such versatility is demonstrated admirably by the authors.

The character of the book as a work of scholarship first emerges in a discussion of the selection of heavy metal compounds for introduction into proteins to provide isomorphous crystalline derivatives. The chemistry of different classes of transition metals and the properties of various categories of complex ion are related to the types of isomorphous substitution which have been obtained. Much of what is offered has not appeared in any review—it is an original arrangement and interpretation of the evidence leading to conclusions which are perfectly orthodox as inorganic chemistry, but which will profoundly change the strategy which protein crystallographers adopt.

Another penetrating analysis deals with an unresolved conflict in the literature between two methods for the exploitation of anomalous scattering effects, due to A. C. T. North and B. W. Matthews. The germ of the idea is traced back to an Oxford thesis by M. Harding; we are led forward to an analysis with no approximations by Singh and Ramaseshan; a tacit assumption in Matthews' choice of the positive value of a square root is pointed out, and his method is clearly related to the "FHLE" method.

In a survey which ranges from film developing technique and microdensitometry to the exploitation of electron diffraction and Mossbauer scattering there are many other stimulating and original insights. The expert

will derive refreshment and pleasure from them. Unfortunately the beginner will be less satisfied. The authors have provided introductory chapters which seem to be aimed at students who know no biochemistry and no crystallography. These chapters make no concessions to the problems of teaching these subjects, and provide an inappropriate *hors d'oeuvre* to the main meal. Some of the obscurities are simply slipshod (that hydrolysis of proteins with boiling mineral acids yields mixtures of up to twenty amino acids), but others will cause serious confusion (the only definition of a Fourier transform is to use it as a synonym for diffraction pattern, for example).

In practice the price of the volume will keep it out of the hands of students, at least in Europe, and it seems a pity that the authors should have misdirected so much effort to cater for them. A good student text is still desperately needed. The book is splendidly up-to-date, including many 1976 references. For some reason the first few pages look rather as though they have been set on a pre-War typewriter, and occasionally one can see evidence of proof corrections, but otherwise the book is well produced. It was a surprise to see several familiar illustrations without acknowledgment.

David Blow

David Blow works at the MRC Laboratory of Molecular Biology, Cambridge, and will shortly become Professor of Biophysics at Imperial College, University of London, UK.

Plutonium and other actinides

Transuranium Nuclides in the Environment. (Proceedings of a Symposium organised by the US ERDA and the IAEA, San Francisco, 1975.) Pp. 724. (IAEA: Vienna; HMSO: London, 1976.) £22.88.

THESE 41 technical papers form an indispensable basis for assessing the "growing concern about man's ignorance of the biogeochemistry of [plutonium and other actinides] . . . in relation to long-term buildup, availability, and transport in the environment" (Noshkin). Nowhere is this concern more apt than the UK, which reportedly discharges to the Irish Sea some 0.3% of its Pu throughput—of the order of 10^4 Ci since 1960.

The message of these papers is clear: our ignorance of pathways is extensive for Pu and is virtually complete for the higher actinides (such as Am) that are more active biochemically and that may

be the dominant hazard. Among the wide range of concentration factors for Pu and higher actinides—from none to several orders of magnitude per trophic level, and from strong discrimination to modest concentration in food chains—exceptions are more common than rules. Unexpected interactions with soil microbes, natural chelates, marine worms, mammalian digestive fluids, and fungi (some of which incorporate actinides into aerial spores) likewise falsify familiar axioms. Particle size is important to these effects, to astonishingly high mobility in soil, to resuspension and adsorption on plant leaves, and to human inhalation (generally a greater hazard than ingestion). Even the chemical behaviour of, say, ^{239}Pu and ^{241}Pu differ markedly. And Pu contamination is indeed ubiquitous: quoted ranges exceed 40 km for routine emissions of a US reprocessing plant, 80 vertical m for bomb residues in coral, and 500 km for dust from Nevada dispersion tests. Both on land and in water, Pu concentrations can be far lower at a point source than at distant sites of favoured deposition.

The most impressive and disquieting example cited is G. L. Meyer's celebrated account of Maxey Flats, Kentucky, a site containing some two-thirds of all US commercial low level nuclear wastes buried during 1963–74. Over 1.6 MCi, including over 80 kg ^{239}Pu and unknown amounts of 300-odd other nuclides, were buried in steel drums and in wood and cardboard boxes. The trenches, in a humid forest underlain by jointed and leaky strata, promptly flooded. Runoff, groundwater, and wind carried contamination off-site (hundreds of metres in a decade) by every 'normal' route plus a few new ones, raising "serious questions about knowledge and concepts of plutonium mobility in the environment"—to say nothing of management. But a British participant, rightly questioning the suitability of the site, was told it seemed preferable to marine discharge, and the marine pathway analyses in other papers hardly contradict this.

The book's attempt to deal not only with actinide pathways but also with the biomedical effects of exposure suffers from too little space and diversity. Although current standards, linearity, neglect of isotopic chemical differences and of gonadal dose, and conventional dosimetry are all properly queried, the brief and truncated reviews hardly do justice to the richness of the controversy. In summary, an important reference work with strong relevance to public policy.

Amory B. Lovins

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obituary

Professor Heinrich Kaiser, the distinguished German spectroscopist, died unexpectedly in Dortmund on August 23, 1976, at the age of 69.

Kaiser was born on February 5, 1907, in Bochum. He studied physics, mathematics and chemistry at Münster, Freiburg and Köln and received his Dr.phil. in 1932. After a time as assistant to F. Försterling, in 1934 he joined the Carl Zeiss Optical Factories at Jena, where he worked on spectrochemical analysis. As a result of collaboration with W. Gerlach, W. Seith and G. Hansen he became involved in the problems associated with the precision of quantitative analysis, its instrumental and other limitations and its mathematical formulation. These problems remained one of his major interests for the rest of his life.

In 1947 he joined the State Institute for Testing Materials in Dortmund, where he set up a spectrochemical laboratory. Because of the lack of modern spectrochemical instrumentation in West Germany at that time, Kaiser set out to convince government and industry of the need for a research institute devoted to chemistry and applied spectroscopy, and in 1952 the 'Institut für Spektrochemie und Angewandte Spektroskopie' was opened in Dortmund with Kaiser as the first director. Kaiser continued to direct the Institute until his retirement in 1975.

Kaiser brought to the direction of the Institute a strong personality and many fruitful policies. Under his guidance, the Institute concentrated on those problems which cannot be solved by industrial or university laboratories alone. He also promoted an interdisciplinary approach, recruiting similar numbers of physicists and chemists for his scientific staff. He arranged 'workshops' for the training of scientists and technicians in new techniques and made welcome in the Institute many visiting scientists from abroad. He played an important role in the English-German cooperation which produced the *Documentation of Molecular Spectroscopy* (DMS). More recently he encouraged the publication of two new spectroscopic atlases: the *UV Atlas* and the *IR-Raman Atlas*. Under his direction the Institute at Dortmund achieved an enviable international reputation and a record of valuable contributions to the science and art of spectroscopy and of service to industry.

Kaiser also continued to make important personal contributions to the

theory of analysis. Himself a physicist, he came to the help of chemists by carefully defining various parameters for analytical procedures in several publications. His definitions of a *complete analytical procedure*, of the *limit of detection*, the *guarantee of purity*, of *selectivity* and *specificity* will probably be accepted as standard definitions in this field.

Kaiser's work received wide recognition. He was Honorary Professor of Physics at Münster University and Ordinarius at Dortmund University. He was president of the Deutscher Arbeitskreis für Spektroskopie and was active in several IUPAC and IUPAP committees. He was also an Honorary Member of the British Society for Analytical Chemistry and the recipient in 1973 of the Hasler Award of the Society for Applied Spectroscopy.

Kaiser had extensive knowledge and great love of music, art, literature and wine which he was happy to share with his friends. This keen interest in the arts manifested itself in many ways. For example he was chairman of the organisation 'Friends of New Arts' and a member of the Administrative Board of the Northwest German Radio. Also through his decisive influence the Institute building at Dortmund is a model of good design showing that functionality need not exclude style.

It pleased him to show parallels in the development of science and the arts. For a concert of chamber music arranged for participants at the memorable XVIth Colloquium Spectroscopium International, over which he presided, in Heidelberg, in 1971, Kaiser found time to write a short essay comparing the development of music from Beethoven's *Septet in E flat major* (1799) to Webern's *Six Bagatelles* (1913) with the progress of physics from Laplace's treatise *Mécanique Céleste* in 1800 to Niels Bohr's theory of the hydrogen atom in 1913! And he even attempted a more direct synthesis of spectroscopy and music. For the opening of the new building for the Institute at Dortmund in 1962 he commissioned a musical composition based on a theme derived by an appropriate scaling down of the frequencies in the Balmer series in the spectra of the hydrogen atom. This composition became known as Kaiser's *Wasserstoff Musik* in affectionate contrast to Handel's *Wasser Musik*.

Kaiser's scholarship and industry will be much missed. Sadly he did not live to celebrate the 25th anniversary of the

Institute at Dortmund in 1977. Spectroscopists in many countries will hope that the Institute will continue to prosper. There could be no more fitting memorial to Kaiser's work.

D. A. Long

Evgeny Konstantinovich Zavoiskii, the distinguished Soviet physicist, died on October 9, 1976. His contributions spanned several fields of research and some thirty years of productive work. He was born in Mogilev-Podolskii, Ukraine, on September 28, 1907, and educated at the Kazan' State University, graduating in 1930. For the next 17 years he worked at the University, becoming Professor in 1945.

In 1944 he was the first person to observe electron paramagnetic resonance, using radio-frequency techniques and iron salts as the absorbing material. This work was carried out in association with S. A. Al'tshuler and B. M. Kozyrev at the Kazan' State University and exploitation of this powerful technique continued there after his departure to the Kurchatov Institute in 1947, the work being celebrated by a Jubilee Conference at Kazan', June 24-29, 1969.

At the Kurchatov he worked initially on solid scintillation detectors and in 1955 reported the first observations of particle tracks using image converters to amplify the light pulses. The Institute became involved in the quest for thermonuclear fusion, and as a consequence his range of interests extended. As a Division Head he was responsible for much of the early work on turbulent heating of plasmas.

In spite of working at the birthplace of the Tokamak, he was interested in other approaches to a viable fusion reactor and in 1968 suggested that relativistic electron beams could provide the energy input needed. This now ranks as one of the five approaches being actively pursued throughout the world.

He was awarded a Lenin Prize in 1957, was elected a member of the USSR Academy of Sciences in 1964 and received many other awards and honours. His contributions will be remembered by a wide range of scientists throughout the world.

R. N. Franklin

Herbert Davenport Kay, CBE, D.Sc., FRS, former Director of the National Institute for Research in Dairying, and Research Professor of Biochemistry in the University of Reading, died peacefully at his home on November 24, 1976, aged 83. He had been an active participant in scientific affairs until a few days before his death.

His academic career, which began in 1914 with graduation from Manchester University with 1st class honours in chemistry, was interrupted by military service throughout the First World War, during which he was twice mentioned in despatches and awarded the Military OBE.

Fortunately in 1919 he decided to resume his biochemical studies. He held two Beit Fellowships and several teaching and research posts in Leeds, London, Cambridge and Freiberg under such eminent scientists as H. S. Raper, Sir Charles Martin, Arthur Harden and Sir Frederick Gowland Hopkins. During this period he was awarded a Ph.D. from Cambridge and a D.Sc. from Manchester. Four years were spent at the University of Toronto as Professor of Biochemistry, until in 1933 he accepted an invitation to become Director of the National Institute for Research in Dairying (NIRD), Shinfield, where he remained until his retirement in 1958.

Professor Kay's early publications reflect a wide range of interests which eventually became centred on problems of phosphorus metabolism. The role of tissue phosphatases in the transport, deposition and mobilisation of phosphorus in health and disease claimed his particular attention. He put the thermolability of milk phosphatase into a practical context when, with his Canadian colleague, W. R. Graham, he devised a simple test for phosphatase in milk, thereby offering a ready means of confirming effective pasteurisation. The test was extremely valuable at that time when raw or inadequately pasteurised milk was likely to contain tubercle bacilli, and after forty years its value is still accepted throughout the world.

The development of the Kay-Graham phosphatase test was characteristic of his commonsense attitude to basic research which, he felt, was vital to the solution of practical problems. As Director of NIRD he saw the need for much more fundamental knowledge in the field of dairy science, and in the years immediately following the Second World War he appointed men and women trained in the basic sciences. He encouraged them further to investigate the physiological and biochemical mechanisms involved in lactation and factors affecting the quality and nutritional value of milk, and to make their findings known to the dairy

farmer and milk processor. He successfully fought against economic restrictions and when he retired in 1958 his dream of establishing a flourishing and well-equipped research institute had been realised.

At 65 he was far from ready to retire. During the next five years he planned and directed the Twyford Laboratories where again his main object was to apply the findings of basic science to industrial problems. From 1963 until his death he retained his interest in research, devoting particular attention to the agricultural uses of copper compounds.

Herbert Kay had served in his youth under several distinguished scientists and by his middle years had every claim to be numbered among them. In 1945 he was elected a Fellow of the Royal Society and the following year was honoured as CBE. His services to the dairy industry were recognised by the award in 1957 of the gold medal of the Society of Dairy Technology. Many learned societies have benefited from his activities and he contributed much to the work of FAO, WHO and UNICEF.

To his staff he was a kindly and encouraging director, recognising no barriers of rank or class, and ready to stand up for his principles against all comers. His familiar figure striding vigorously across the Institute's fields will be remembered by many with affection. His happy family life with his wife and four sons did much to sustain him in his demanding professional duties. **Marie E. Coates**

Dr Henry Miller, Vice Chancellor of the University of Newcastle upon Tyne, and formerly Dean of the Medical School and Professor of Neurology, died on August 25, 1976, at the age of 62.

Obituaries in the national press and the national medical journals have justly paid tribute to Dr Miller's various talents, in particular his generosity and wit, his brilliant mind and profound intelligence. Comment has been made on his energy and his literary output which always more than justified his hedonistic tendencies.

Following qualification in Medicine and a period as Research Fellow at Johns Hopkins Hospital in Baltimore, Henry returned to the United Kingdom to have his career interrupted by war-time service in the Royal Air Force. He then trained further at Hammersmith and at the National Hospital, Queen Square, before his years as a successful part-time general physician at Newcastle's teaching hospital.

Although he was productive in these early years writing many papers and editing *Progress in Clinical Medicine* through many editions, his academic and research career began with the founding of the University Department of Neurology in the mid-50s. Having attracted to that Department neuro-radiologists, neurosurgeons and neurological colleagues, he developed an active investigative team of research and ancillary workers from whom there flowed a succession of scientific papers on many diverse aspects of basic and clinical neurology. His major interest became the demyelinating diseases and with scientific collaborators he made major contributions to our understanding of the epidemiology, diagnosis and management of multiple sclerosis.

He was appointed to a personal Chair in Neurology in 1964 and became Dean of the Medical School in 1966 and Vice Chancellor of the University of Newcastle in 1968.

He was always prepared to admit his lack of talent for the physical aspects of bench research and never claimed to be a laboratory worker. Such was his appeal, however, that scientists both junior and senior sought to work within his department and were nurtured and stimulated by the flow of ideas and the enthusiasm which stemmed from this very original mind. His major writings extended from demyelinating disease through the problem of accident neurosis to the more general topics of the country's Health Service and of Medicine in relation to community life both at home and abroad.

He was much honoured, and others have listed his many achievements which included his invitation as Visiting Professor to many overseas universities, Secretary General of the World Federation of Neurology, one-time President of the Association of Physicians of Great Britain and Ireland, and latterly a very significant member of the Committee of Vice Chancellors.

His literary contributions were extensive, but perhaps amongst the most provocative were those he produced in his last few years, in particular his monograph on *Medicine and Society* and his series of articles in *The Listener*.

Whilst not always agreeing with the ideas he expressed, none failed to admire his style, and in the lecture room, television studio, or in private, his personality never failed to attract and hold his audience.

His behaviour was extravagant but always he challenged his friends and colleagues to justify the principles which they held, a challenge some found irreverent but others essentially stimulating.

J. B. Foster